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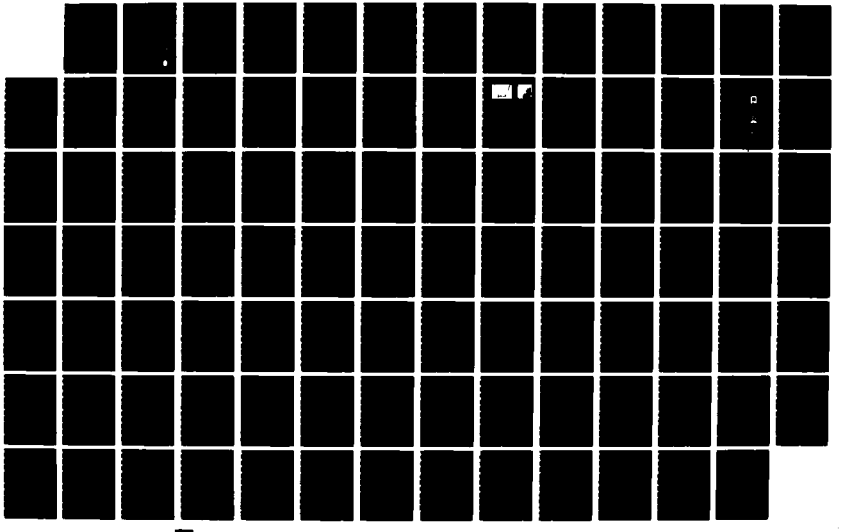

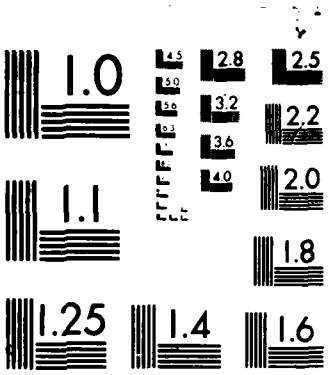

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USAFSAM-TR-86-44

BIOLOGICAL EFFECTS OF MILLIMETER-WAVE IRRADIATION

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Brooks Air Force Base, TX 78235-5301

NOTICES

This final report was submitted by the Department of Electrical Engineering, University of Utah, Salt Lake City, Utah 84112, under contract F33615-84-C-0610, job order 2312-W1-17, with the USAF School of Aerospace Medicine, Human Systems Division, AFSC, Brooks Air Force Base, Texas. Dr. David N. Erwin (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

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The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Experiments were conducted to verify the reported high degree of sensitivity of growth rates of yeast cultures to frequency of millimeter-wave irradiation in the band 41.650 to 41.798 GHz. A new irradiation chamber was designed and built to allow simultaneous irradiation and sham irradiation of recirculating suspensions of Saccharomyces cerevisiae that were maintained with a temperature difference of less than 0.01°C. No difference larger than ± 4% was ever detected in the growth rates at any of the highly stabilized (within ± 50 Hz) irradiation frequencies for which the effects had been reported by earlier workers. Experiments were also performed to determine the Raman spectra of cultures of Bacillus megaterium to investigate if these are dependent on the stage of their life cycle as reported by Webb et al. The results were negative. A further study to investigate the ability of millimeter waves to induce conformational changes in lipid bilayers of dipalmitoyl-

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19. ABSTRACT (Continued)

phosphatidylcholine (DPPC) liposomes below and above the transition temperature of 41°C also gave negative results. For these experiments the conformational characteristics of the liposomes were evaluated using Raman spectra with and without millimeter-wave irradiation at 41.650 GHz.

A computer-controlled measurement system has been set up to determine the complex permittivities of biologically relevant samples in the frequency band 26.5 to 60 GHz. The measured data are given for deionized water, 10% saline, human blood, 20% bovine serum albumin, preparations of Saccharomyces cerevisiae with concentrations of 10^7 and 10^9 cells/mℓ, and for the skin of the palm of a human subject.

BIOLOGICAL EFFECTS OF MILLIMETER-WAVE IRRADIATION

INTRODUCTION

The increased use of millimeter waves for civilian and military purposes has generated great interest in the possible health hazards of millimeter-wave radiation at thermal and nonthermal levels [1]. Also, the presence of several reports indicating that biological effects could be induced by low-level radiation (<5 mW/cm^2) has stimulated interest in the more basic question of the mechanisms of interaction of millimeter waves with biological systems.

This project has approached the question of the biological effects of millimeter waves on several fronts: independent verification of the reported frequency-sensitive growth rates of yeast cultures to millimeter-wave radiation [2-4], Raman spectroscopic studies of bacterial cell suspensions and of a model of cell membranes [liposomes], determination of dielectric properties of biologically relevant samples, and experiments to determine the presence of surface acoustic waves in polystyrene microspheres and liposomes.

EFFECTS OF MILLIMETER-WAVE IRRADIATION ON GROWTH OF SACCHAROMYCES CEREVISIAE

The growth of Saccharomyces cerevisiae cultures has been reported [2-4] to be strongly influenced by millimeter-wave irradiation around 41.7 GHz. In particular, a change of only 8 MHz in the irradiation frequency was reported to be sufficient to alter significantly the magnitude of the effect.

The need to verify such extreme sensitivity of yeast cells has motivated this section of the project. A new irradiation chamber was designed and built to allow simultaneous irradiation and sham irradiation of recirculated cell suspensions. The temperature difference was < 0.01°C and the growth was measured optically. A klystron, phase-locked by an EIP-578 microwave counter, was used to generate millimeter waves stable to within ± 50 Hz for the 4 h of irradiation and the frequency was measured with up to 11 significant digits. At least three experiments were done at each frequency. The irradiation frequencies were in the ranges, between 41.650 and 41.798 GHz, in which the

largest effects were previously reported [2-4]. In these regions the frequencies were 2 MHz apart.

No difference larger than ± 4% was detected in the yeast growth rate at any of the irradiation frequencies. Furthermore, these effects were not significant at the 95% confidence limit. Plate counts performed at the end of each experiment were in fair agreement with the growth data. A complete report on these experiments has been published [5] and its reprint is attached as Appendix A.

RAMAN SPECTROSCOPY OF LIPOSOMES EXPOSED TO MILLIMETER WAVES

Cultures of Bacillus megaterium were reported to show Raman spectra dependent on the stage of their life cycle [6, 7]. A preliminary study was conducted to investigate the spectral features of Raman spectra of B. megaterium cells after heat and cold shocks, as indicated in references 6 and 7. No biologically related features were detected in the Raman spectra at any stage of the cell cycle. These results have been published [8, 9], and their reprints are attached as Appendixes B and C.

Even if Raman spectra had been detected for cultures of bacterial cells, it would have been rather difficult to ascribe their origin to a particular structure of molecular process. We, therefore, decided to use another approach to study the possible mechanism of interaction of millimeter waves with biological systems. A relatively simple and well-characterized model of cytoplasmic membranes has been used; and Raman spectroscopy was used to prove, concurrently with the irradiation, the ability of millimeter waves to induce conformational changes in lipid bilayers. Experiments were performed on dipalmitoyl-phosphatidylcholine (DPPC) liposomes below and above the transition temperature, 41°C, of DPPC. As expected, a temperature increase induced spectral changes, while no differences were detected upon millimeter wave irradiation. A complete report on these experiments has been published in the Journal of Applied Physics [10]. A reprint copy of reference 10 is attached as Appendix D.

BRILLOUIN SPECTROSCOPY OF LIPOSOMES AND MICROSPHERES

It is possible that surface acoustic waves may be supported on microspheres and also on liposomes. To test this hypothesis, preliminary experiments have been performed with Brillouin spectroscopy using a Fabry-Perot interferometer in single and five-pass configurations. The spectra of $CHC\ell_4$ and of water are shown in Figures 1 and 2. These spectra were obtained by accumulating 100 spectra on 1024 channels with 2-ms accumulation/channel. The laser source was set at 488.8 nm and an etalon was placed in the laser. Experiments are presently underway to determine the presence of surface acoustic waves in microspheres and liposomes.

EXPERIMENTAL DETERMINATION OF COMPLEX PERMITTIVITIES OF BIOLOGICAL SAMPLES

We have developed a computer-controlled measurement system operating at frequencies from 26.5 to 60 GHz. The measurement system is schematically illustrated in Figure 3. The key components of the measurement system are a precision slotted line, a diode detector for standing-wave measurements, a frequency counter EIP-578, and HP 86B computer for overall automation. The traveling probe of the slotted line is moved in steps of 0.3 mm by a computer-controlled stepping motor. A large dial indicator calibrated in 0.01-mm increments gives the position of the probe. At each location of the slotted line probe, the standing-wave voltage is measured by the diode detector (sensitivity: - 60 dBm) via SWR meter, A/D converter and HP 86B computer. By averaging 10 or more repetitive measurements of standing-wave voltages, the extreme variation was limited to ± 0.06 dB. The standing-wave voltages were measured at 40 equally spaced positions of the slotted line probe. The phase and magnitude of the reflection coefficient were obtained by implementing the three-probe reflectometer formulation given by Caldecott [11]. Between 20 to 35 independent values of the phase and magnitude of the reflection coefficient are obtained due to different combinations of the standing-wave voltages. The accuracy of the reflection coefficient measurements is improved significantly by averaging these reflection coefficient values.

Using the measurement system of Figure 3, the complex permittivities were measured for several samples; e.g., 10% saline solution, human blood, 20%

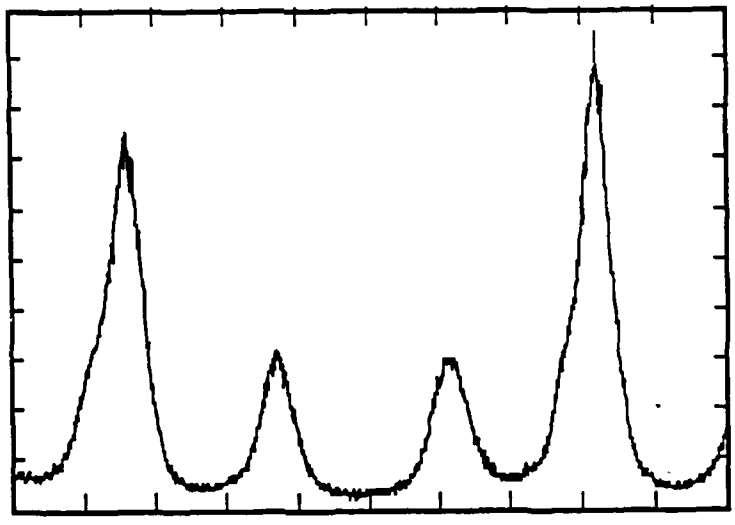

Figure 1. Brillouin spectrum of $CHC\ell_4$ (single pass, 100 scans, 2 ms/channel). Free spectral range ≈ 18.7 GHz.

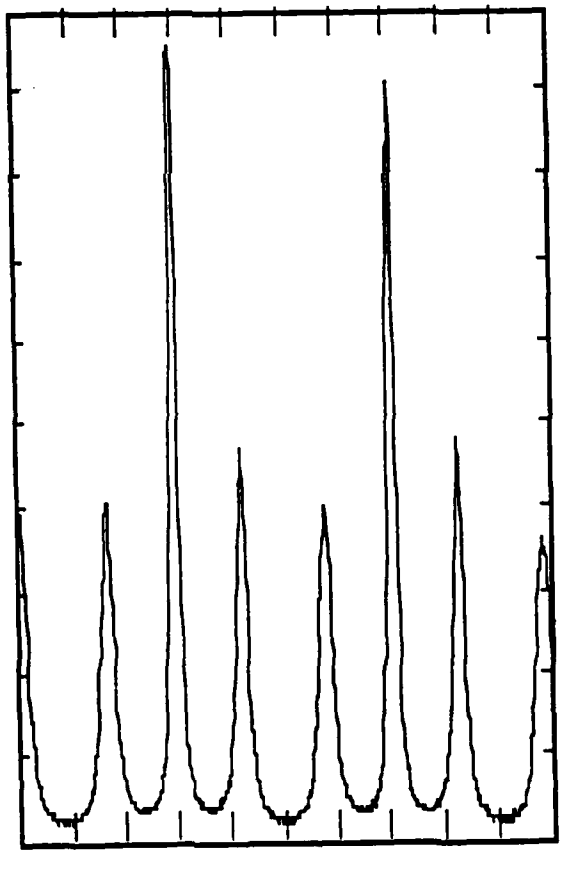

Figure 2. Brillouin spectrum of deionized water (single pass, 100 scans, 2 ms/channel). Free spectral range ≈ 18.7 GHz, phonon shift frequency ≈ 5.6 GHz.

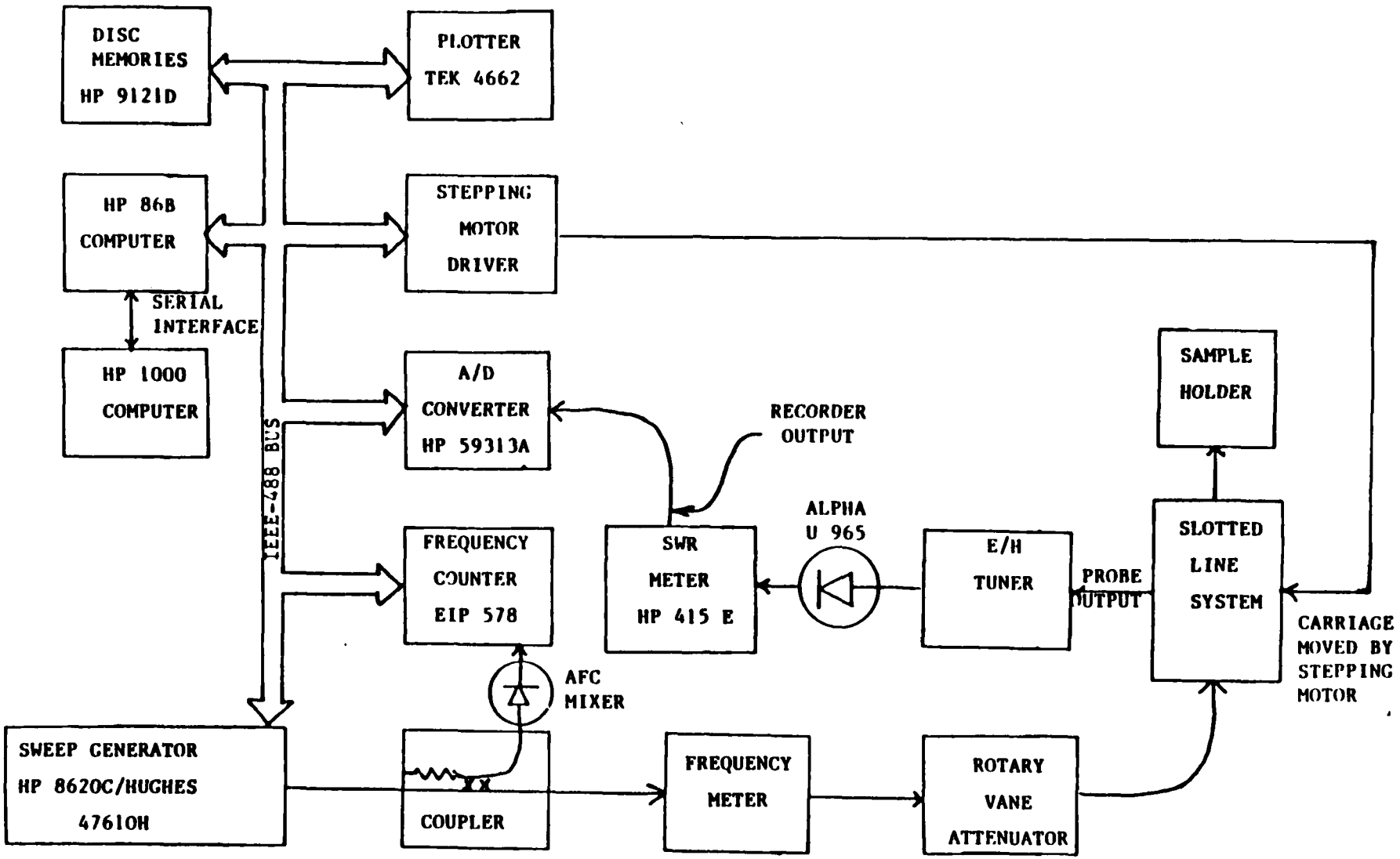

Figure 3. Block diagram of automated complex permittivity measurement system.

bovine serum albumin (BSA) solution and, preparations of Saccharomyces cerevisiae with densities of 10^7 and 10^9 cells/mℓ. The measurements were not performed in the frequency range of 35 to 40 GHz because of very low output from the tunable probe of the slotted line. Also, in the frequency range of 46 to 50 GHz, the complex permittivity data were not obtained because of problems with the stability of IMPATT diode oscillator.

A modified "infinite" sample method has been developed to determine the dielectric properties of the biological samples. In this method, an impedance-matching transformer is used to match an electrically infinite biological sample to air-filled waveguide. The matching transformer consists of a $\lambda_{g\varepsilon}/4$ or $3\lambda_{g\varepsilon}/4$ waveguide section of a suitable low-loss dielectric material. The choice of the matching transformer material would depend on its dielectric constant, loss tangent, and machinability. For biological samples, it has been found that low-loss materials with dielectric constant between 6 to 10 are suitable. The modified infinite sample method has several advantages over other methods (e.g., Von Hippel's short-circuit technique, microwave bridge method, etc.), including (1) elimination of tedious sample preparation associated with submillimeter sample lengths, and (2) relatively easier broadband measurements.

The frequency ranges 28 to 33 GHz, 42 to 45 GHz, and 52 to 57 GHz have been covered by three matching transformers of alumina of lengths $\lambda_{g\varepsilon}/4$, $3\lambda_{g\varepsilon}/4$, and $5\lambda_{g\varepsilon}/4$, respectively. From uncertainty analysis, it has been observed that ± 10% accuracy in complex permittivity of a biological sample can be obtained if the dielectric constant and the length of the alumina matching transformer are known to within ± 1% and ± 5 μm, respectively. Because these types of accuracies cannot be realized by conventional methods, it is difficult to characterize the matching transformer separately. This problem has been alleviated by estimating the matching transformer parameters from measurements with liquids of known dielectric properties; e.g., deionized water. The measured complex permittivities of deionized water having a conductivity of 200 μS/m are shown in Figure 4 with the best-fit Debye model (solid line). The best-fit Debye parameters (ε_s = 78.65, ε_∞ = 4.04, and τ = 8.38 ps) are in fairly good agreement with those obtained from Schwan et al.

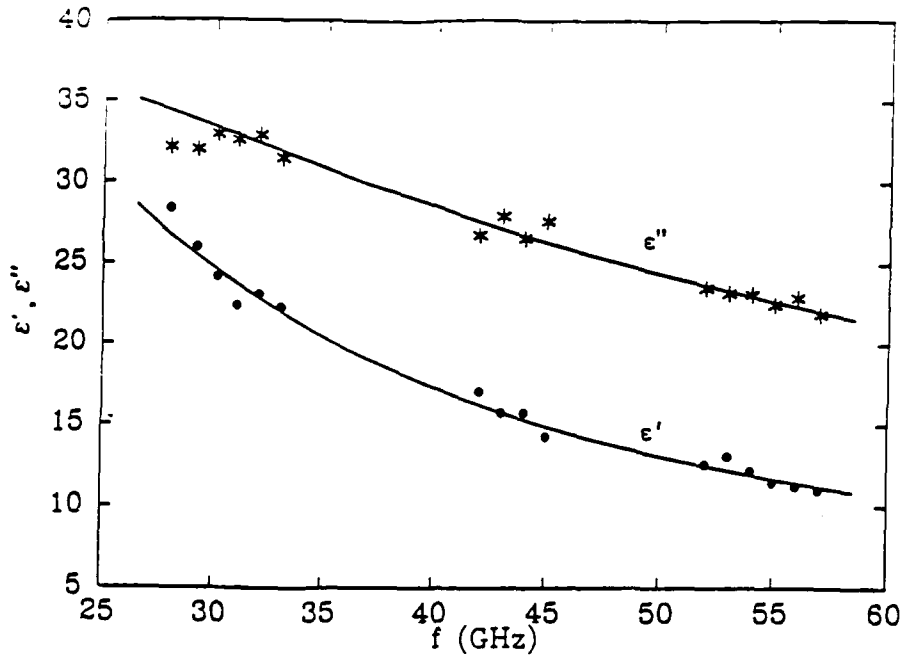

Figure 4. Complex permittivity of water.

[12]. The estimated dielectric constant of alumina matching transformer from deionized water measurements is 9.25 which is in close agreement with 9.15 ± 0.16 measured using Von Hippel's shorted line technique [13]. The estimated lengths of the matching transformers also agree closely with the physically measured lengths.

The measured complex permittivity data, along with the data from best-fit Debye model, are shown in Figures 4-9 for 10% saline, human blood, 20% BSA solution, and Saccharomyces cerevisiae cell suspensions of densities 10^7 and 10^9 cells/ mℓ. The Debye equation is given by

$$\varepsilon^* = \varepsilon_\infty + \frac{(\varepsilon_s - \varepsilon_\infty)}{1 + j\omega\tau} - \frac{j\sigma}{\omega\varepsilon_o} \qquad (1)$$

where, ε_s and ε_∞ are the static permittivity and infinite-frequency permittivity, respectively. σ and τ represent ionic conductivity and relaxation time, respectively. The Debye parameters (ε_s, ε_∞, τ and σ), along with standard deviations, are given in Table 1 for different biological materials

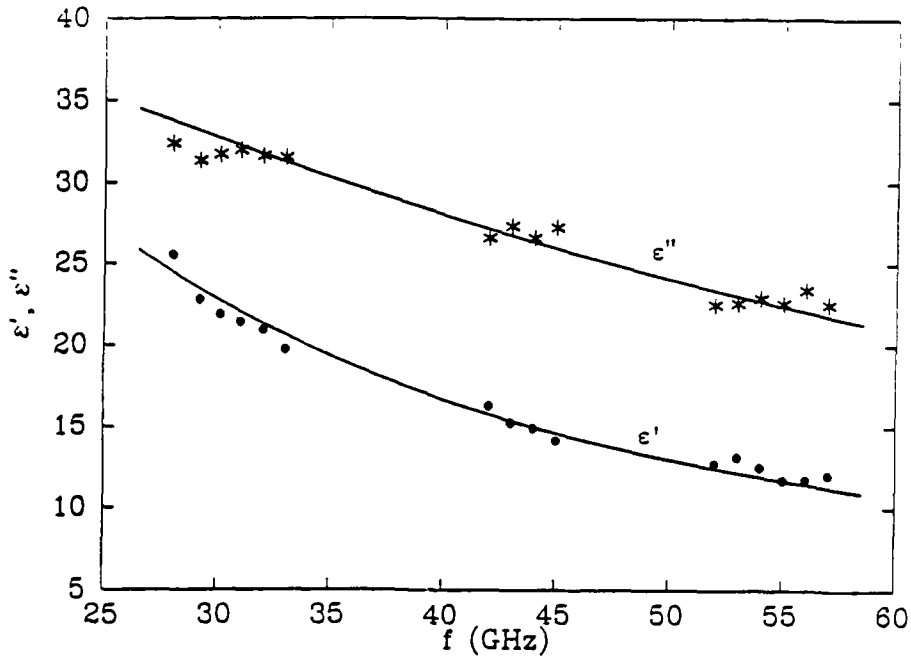

Figure 5. Complex permittivity of 10% saline.

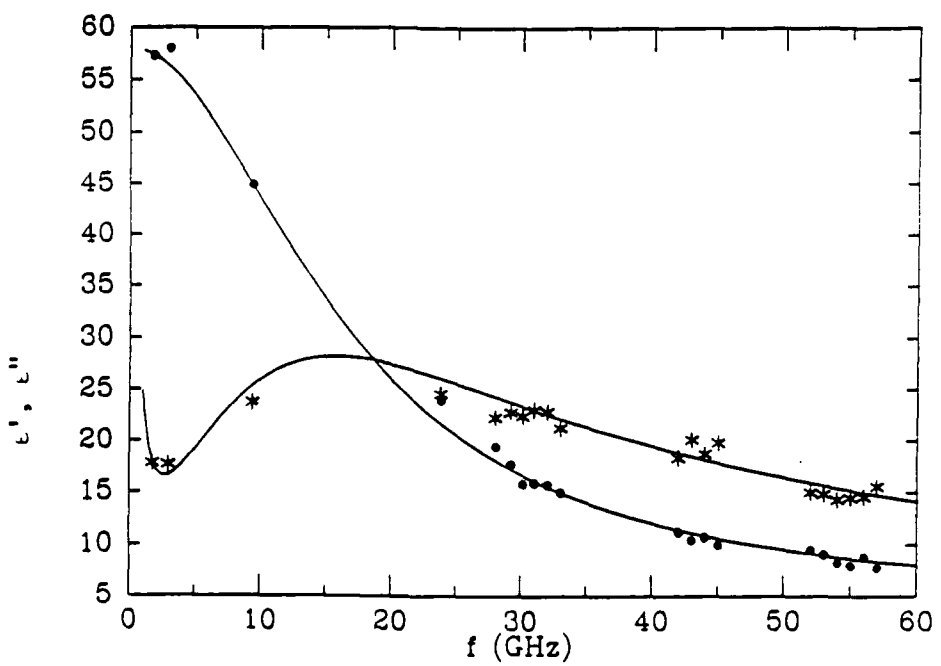

Figure 6. Complex permittivity of blood.

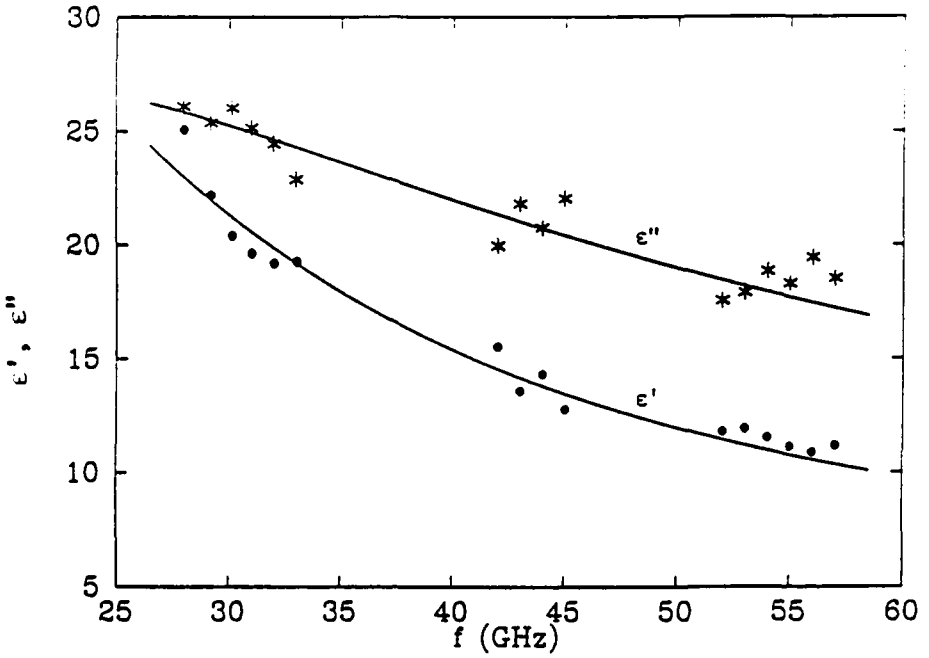

Figure 7. Complex permittivity of 20% BSA solution.

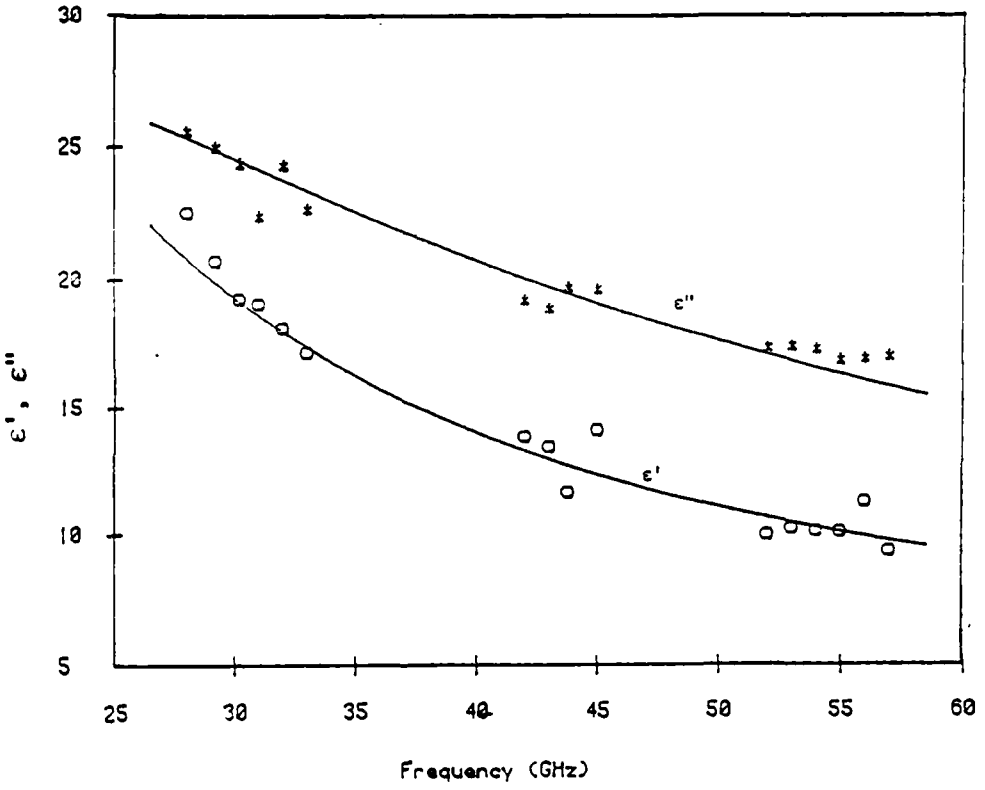

Figure 8. Complex permittivity of S. cerevisiae (density = 10^9 cells/mℓ).

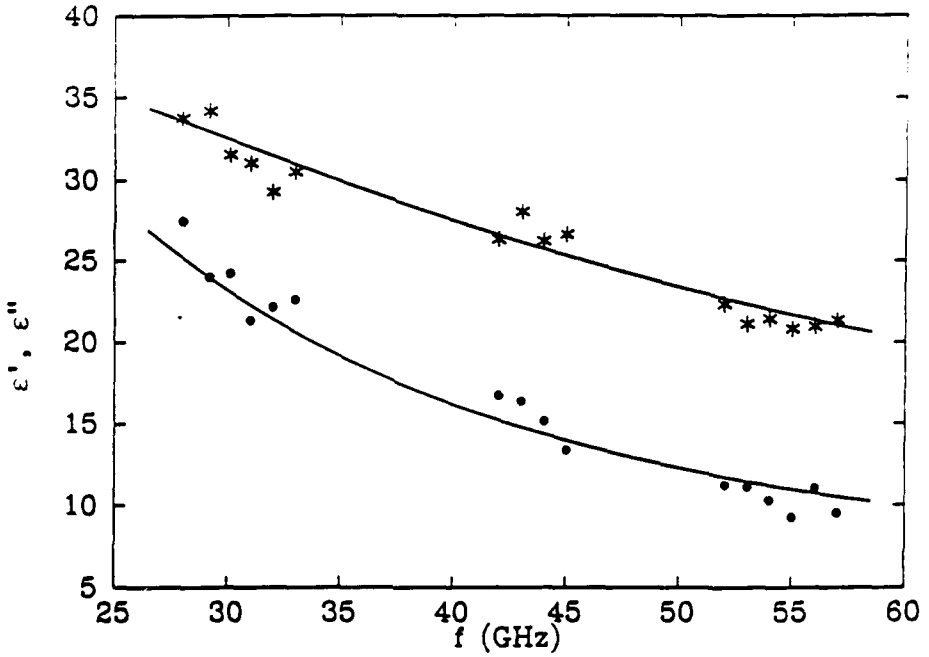

Figure 9. Complex permittivity of S. cerevisiae (density = 10^7 cells/mℓ).

TABLE 1. DEBYE PARAMETERS FOR VARIOUS BIOLOGICAL MATERIALS

Sample	Temp (°C)	ε_s	ε_∞	τ (ps)	σ (S/m)	RMSE
Water	23±1	78.7±1.1	4.04	8.58±0.14	0.0	.56
10% saline solution	23±1	55.9±0.9	4.35	7.10±0.13	13.4	.64
Human blood	23±1	58.0±1.6	4.24	9.64±0.24	1.2	.91
Bovine serum albumin 20% C. V.	23±1	58.5±1.4	4.17	7.80±0.19	0.0	.88
S. cerevisiae 10^9 cells/mℓ	24±1	61.0±1.7	4.90	9.06±0.28	0.0	0.77
S. cerevisiae 10^7 cells/mℓ	24±1	78.3±2.0	4.00	9.00±0.3	0.0	1.10
Human skin in vivo	—	28.5±1.3	1.50	10.05±0.6	0.0	.57

that have been tested. The root mean square error (RMSE) given in Table 1 is defined as

$$RMSE = \left[\frac{\sum_{i=1}^{N} \left| \varepsilon_{mi}^{*} - \varepsilon_{i}^{*} \right|^2}{2N} \right]^{1/2} \qquad (2)$$

where ε_{mi}^{*} and ε_{i}^{*} represent measured complex permittivity and complex permittivity from the Debye equation for ith frequency, respectively, and N is the number of frequencies.

MEASUREMENT OF THE COMPLEX PERMITTIVITY OF HUMAN SKIN IN VIVO

The computer-controlled measurement system described in the last section was used for computer permittivity measurements of the human skin in vivo. The complex reflection coefficient was measured by pressure contact of the waveguide flange against an area of the body, mostly the palm of the hand. The high reflectivity of the human skin was reduced by using a quarter wavelength matching transformer of Teflon. Complex permittivities were determined from measured reflection coefficients using a variational method iteratively for calculating the input impedance. A zero-finding technique algorithm was used for optimization.

Figure 10 shows the real and imaginary parts of the permittivity for the skin of the palm along with the values obtained from the best-fit Debye equation. Equation 1 for the Debye relaxation model was fitted to our data for the frequency band 26.5 - 60 GHz and to the lower frequency data reported by Hey-Shipton et al. [14]. The Debye parameters for the skin of the palm are also given in Table 1.

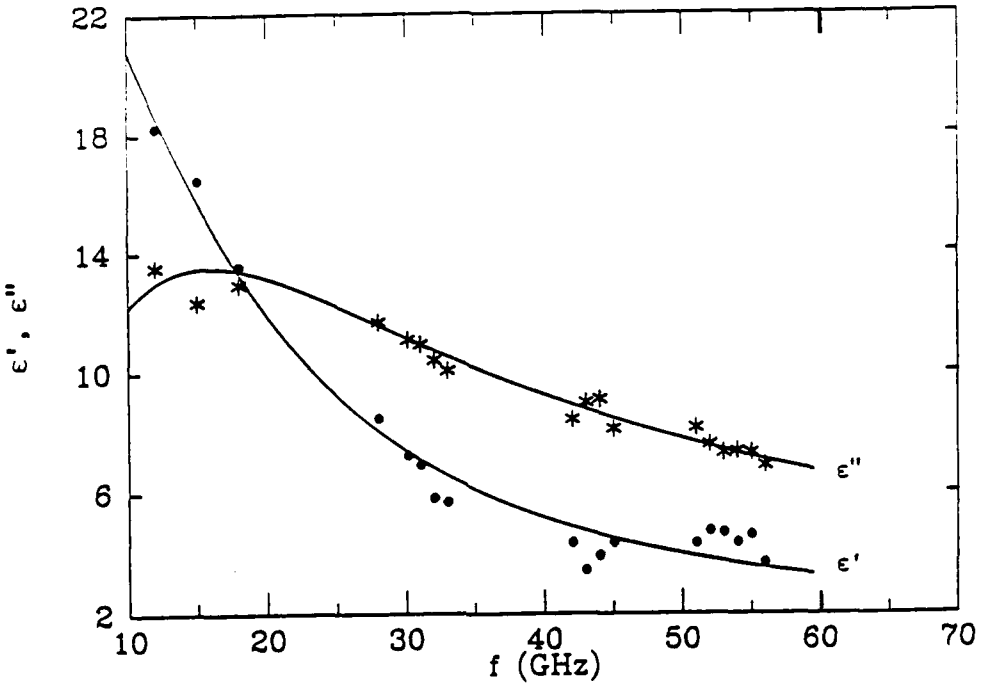

Figure 10. Complex permittivity of the skin of the palm.

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IEEE TRANSACTIONS ON BIOMEDICAL ENGINEERING, VOL. BME-33, NO. 11, NOVEMBER 1986

Effect of Millimeter-Wave Irradiation on Growth of *Saccharomyces cerevisiae*

LUCIANO FURIA, MEMBER, IEEE, DOUGLAS W. HILL, AND OM P. GANDHI, FELLOW, IEEE

Abstract—**Cultures of *Saccharomyces cerevisiae* were exposed for 4 h to millimeter waves in three frequency ranges between 41.650 and 41.798 GHz. The irradiation frequency was stabilized to within ±50 Hz. The temperature difference between irradiated and sham-irradiated samples was maintained to within ±0.01°C. Growth was measured optically during the irradiation, and viability counts were done at the end of the irradiation. At least three experiments were performed at each of 15 frequencies.**

No differences larger than ±4 percent were detected in the growth rates at any of the selected frequencies. Such differences were not significant at the 95 percent confidence limit. Results obtained with plate counts correlated favorably with the optical absorbance data. While our data are in contrast with those reported from other investigators, these experiments support conclusions of our previous studies, and of some other investigators, showing that, under strictly controlled conditions, no statistically significant nonthermal effects can be induced by millimeter-wave irradiation of a variety of prokaryotic and eukaryotic cells.

INTRODUCTION

SINCE 1968 several investigators have reported a variety of biological effects induced by irradiation with millimeter waves (mm-waves). Many have further claimed strong dependence on the irradiation frequency. Representatives of these findings include effects on growth rates of *Rhodotorula rubra* [1], *Saccharomyces cerevisiae* [2]–[6] *Escherichia coli* [7], [8], *Candida albicans* [1], [9], reduced viability in *Sacch. cerevisiae* [10], protection of rabbit bone marrow cells from X-ray damage [11], increased colicin [12] and lambda phage induction in *E. coli* [13], and puffing of giant chromosomes of *Acricotopus lucidus*, an insect [14].

Some reports indicated changes in the magnitude of the effect when the frequency was shifted by a few megahertz. Furthermore, some reports were characterized by the low power densities needed to induce the effect: a power density as low as 10 μW/cm^2 was reported to effect colicin induction in *E. coli* [12]. An extensive review of the published literature can be found in [15].

Unfortunately, many of the above mentioned reports lack essential details of the experimental procedure and data, making independent duplication or evaluation quite difficult. Some of them, however, have been independently repeated, mostly with negative findings. Some of the negative experimental results include: no effects on growth rate of *E. coli* [16] and no changes in viability of *Sacch. cerevisiae* [17]. No evidence of cytological effects as determined by electron microscopy [18], and in protein synthesis [19] were observed in BHK-21 cells exposed to 202 frequencies in the *E*- and *U*- band at various power densities. Finally, no statistically significant effects were found on colicin induction [20], [21], on mutation rates of *Salmonella typhimurium* or on induction of lambda phage in *E. coli* by *U*- and *E*-band mm-wave irradiation [22], [23].

A strong dependence on irradiation frequency of the growth rate of yeast suspension has been reported independently by two research groups. Devyatkov [1] reported increases up to +30 percent in the growth rate of *R. rubra* exposed for 15 h at 7.18 mm (41.783 GHz) and a decreased growth rate of up to −40 percent when exposed at 7.19 mm (41.725 GHz). However, no indications were given about experimental procedures such as irradiation conditions, handling of the controls, temperature control and measurement, frequency stability, and power density.

Grundler, Keilmann, and co-workers [2], [6] have also reported that the irradiation of *Sacch. cerevisiae* in the 41.650–41.800 GHz range, showed both growth enhancement (up to +15 percent) and growth inhibition (down to −13 percent) frequency regions. Frequencies exhibiting significantly different effects were reported to be only 8 MHz apart. The data suggest that the yeast suspensions showed a sensitivity to frequency changes of less than 1 part in 5000. Due to this reported frequency dependence, this phenomenon has been termed a "resonant effect" [2]–[6].

Although such reported frequency sensitivity has stimulated interest in the possible mechanism and site(s) of action of mm-waves [18]–[23], [24], [25], it is essential to establish the validity of these observations and their interpretation. In the following we report on our experiments designed to study further the possible effects of mm-waves on the growth of *Sacch. cerevisiae*.

MATERIALS AND METHODS

A. Description of the Irradiation Experiment

The organism used in this study was obtained from the University of Utah stock collection, grown on Sabouraud

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glucose agar (Neopeptone® 10 g/l, glucose 40 g/l, Bacto agar® 15 g/l) stored at 4°C and passed every month. From these cultures, organisms were inoculated in Sabouraud glucose broth (Neopeptone® 10 g/l, glucose 20 g/l, pH 5.6) and incubated overnight at 32°C on an orbital shaker (40–50 rpm). The overnight culture was diluted 1:400 and incubated at 32°C for 2 h on an orbital shaker before loading each of two irradiation circuits with 2.5 ml of the suspension. The irradiation and sham irradiation were started after another hour of incubation in the circuits so as to irradiate the yeast during the log phase of growth. The temperature difference between irradiated and sham-irradiated suspensions was maintained within ±0.01°C using a nichrome heater placed under the sham-irradiated sample holder. The suspensions were recirculated with a peristaltic pump LKB 2115 with a flow rate of 7 ml/min. The experimental temperature was 32.0 ± 0.1°C. The temperature coefficient of the growth rate was measured between 30 and 34°C and was 0.024 h^{-1}/°C. The mean growth rate at 32°C was 0.595 h^{-1}, corresponding to a duplication time of 70 min.

The irradiation time was 4 h with power in the waveguide set at 20.0 ± 0.5 mW. Due to the tapered design of the sample holder, the power coupling of mm-wave radiation to the suspension was larger than 99 percent as estimated from reflected power measurements. Even though we used continuous wave (CW) microwaves, circulating the suspension did, in fact, provide a modulation of sorts with a period of 30 s and a duty cycle of 0.06.

Small flow-through cuvettes were placed within the recirculating circuits to allow turbidity measurements. For both the irradiated and sham-irradiated samples, triplicate sets of data were collected at the beginning of the irradiation and hourly thereafter. All the data collection, storage, and analysis was performed with an IBM personal computer. Absorbance values at the observation time intervals were analyzed with a regression analysis program to fit an exponential curve. The output of the program gave the estimated mean values of growth rates μ_i and μ_r for irradiated and sham-irradiated suspensions. The program also indicated the 95 percent confidence limits of the estimated growth rates for each experiment. A typical exponential growth curve, measured optically, for an experiment without either mm-wave radiation or compensatory electric heating is shown in Fig. 1.

Plate counts were done at the end of the irradiation experiment using three 0.2 ml aliquots from each suspension. After tenfold serial dilution, the suspensions were plated in quadruplicate and colonies counted after 48 h of incubation at 32°C. Dilutions were chosen to give approximately 200 colonies per plate.

B. Microwave System

As mentioned previously, the main feature of a so-called "resonance effect" is its strong dependence on the irradiation frequency. Therefore, some of the basic requirements for observing such an effect should be 1) high frequency stability within a single experiment, 2) high precision in the frequency resetting from experiment to experiment, 3) high accuracy in the absolute frequency determination, and 4) low noise and narrow bandwidth of the source. After testing various mm-wave sources such as free running klystrons and IMPATT diodes, we chose a klystron, Varian model VA302-BT, phase-locked by a source-locking microwave counter, EIP model 578. By using a highly stable quartz crystal enclosed in a temperature controlled oven, the rated frequency stability of the counter was ±50 Hz for a center frequency around 40 GHz. This stability was for at least 4 h, the typical length of an experiment. The absolute frequency could be measured with up to 11 significant digits. The rated aging of the reference quartz crystal is claimed by the manufacturer to give a long-term accuracy (1 year) of the absolute frequency of less than ±8 kHz. The residual half-power bandwidth was 40 kHz when phase-locked, as measured with a Tektronix 491 spectrum analyzer.

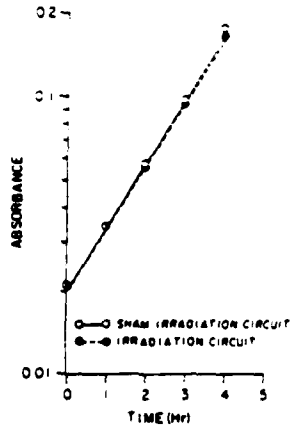

Fig. 1. A typical growth curve of *Saccharomyces cerevisiae* suspension measured optically in the absence of mm-waves. For this particular experiment the growth rate of the "sham-irradiation" was 0.531 h^{-1}, while for the "irradiation" circuit it was 0.522 h^{-1}. The 95 percent confidence limits were about ±0.015 h^{-1} (±2.5 percent). The points represent the average of three measurements taken at the indicated time intervals, the lines represent the fitted curves calculated with an exponential regression program. The cell concentrations at the start and at the end of an experiment were in the 6.5×10^5–1.1×10^6 and 7×10^6–1.2×10^7 cell ml ranges, respectively. The time intervals and dilution factors were chosen so as to do experiments only during the log phase of the yeast growth and to use the linear response region of the photometric system.

The CW power in the waveguide was measured with a thermistor, Hughes model 4489H, connected to a power meter, Hewlett-Packard model 432A. A variable attenuator was inserted before the thermistor to cut off, from time to time, the microwave power to the thermistor, therefore allowing correction for possible thermal drift of the thermistor. The sample holders were mounted at a 6° angle directly on the waveguides (Fig. 2) thereby providing enough tapering to ensure a measured power coupling of about 99 percent. It was confirmed during preliminary tests that the reflected power was always less than 1 percent, regardless of the irradiation frequency. The fixed geometry of the sample holder did not require routine measurement of the reflected power. A complete block diagram of the microwave circuit is shown in Fig. 3.

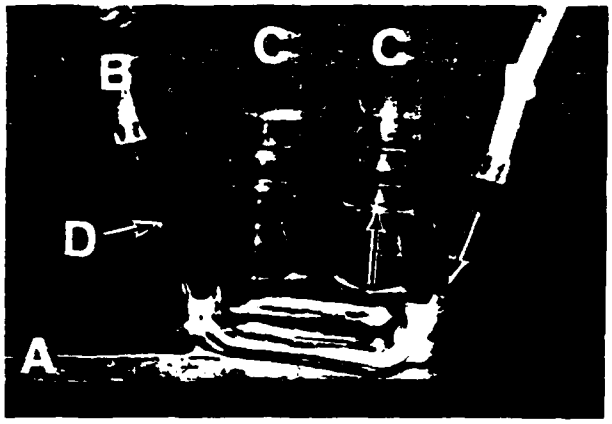

Fig. 2. Sample holder used for irradiation. The Teflon® coated thermocouples were inserted in the suspensions through holes in the silastic rubber tubing. *A*—WR-19 waveguide; *B*—cell suspension circuit; *C*—water jacket circuit; *D*—thermocouple for the temperature measurement of the sample at the exit of the sample holder. Another thermocouple was placed also at the sample holder inlet. The directions of flow are indicated by the arrows on the circuits. The nichrome heater was placed between the waveguide and the sham-irradiation sample holder.

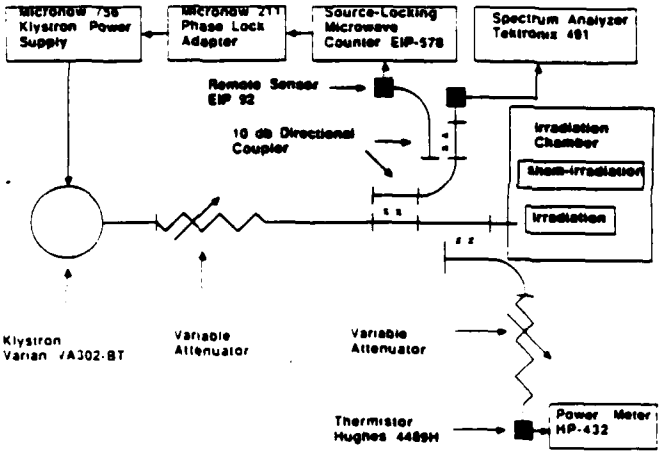

Fig. 3. Block diagram of the microwave system. The section necessary for the reflected power measurement was removed during the actual experiments.

C. The Irradiation Chamber

The chamber was made with an aluminum box lined with polyurethane foam. On the bottom of the chamber we placed a copper tank in which temperature controlled water was recirculated at the rate of 10 l/min. The water was circulated from a water bath kept at 32.0 ± 0.1°C by a YSI 72 proportional temperature controller and a solid-state cooler (Whirlpool). The waveguides with the sample holders were bolted on the copper tank. To obtain a uniform temperature distribution we found it essential to place an electric fan inside the chamber. The 60 Hz magnetic field generated by this fan did not differ from the background intensity, 0.03 G, at either the sample holder or the cuvette location. A top view of the irradiation chamber is shown in Fig. 4. The glass sample holder had a volume of 0.25 ml (2.5 × 0.5 × 0.2 cm) and was connected to the peristaltic pump with silastic rubber tubing (1.3 mm i.d.). Water jackets were placed around the glass sample holders to improve the thermal exchange. Water for the water jackets was circulated at a rate of 1 l/min, from the same water bath supplying the copper tank. Teflon® coated thermocouples (Bailey-Sensortek IT-18) were placed in the suspensions at the inlets and outlets of the sample holders. These were connected to a digital differential thermometer (Bailey-Sensortek TH-6D) that allowed a rated precision in the absolute mode of ±0.1°C and of ±0.01°C in the differential mode. The temperature difference was minimized by adjusting the current in the nichrome heater. No temperature increase due to the microwave or electrical heating was detected in the suspensions throughout the experiment. The use of small cuvettes, HELLMA 144 O-NS, and the placement of the head of the peristaltic pump inside the irradiation chamber allowed the use of a total suspension volume of 2.5 ml.

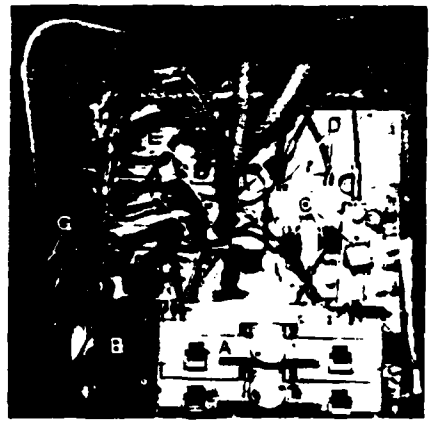

Fig. 4. Top view of the irradiation chamber. The copper tank is at the bottom of the chamber. A metallic lid was placed on the chamber during the experiments. *A*—peristaltic pump; *B*—electric fan; *C*—cuvettes for turbidity measurements; *D*—optical fiber bundles; *E*—sham-irradiation sample holder; *F*—irradiation sample holder; and *G*—temperature probe for water jackets.

D. Optical System

A fiber optic system was used to measure the turbidity of the yeast cultures and in turn the growth rates of the organisms. We used optical fibers made with Crofon (Dupont) fiber bundles (48 fibers, each 0.25 mm in diameter). An interference filter (Edmund Scientific Corporation, 650 nm center wavelength, 10 nm bandwidth) was placed between the optical fibers and a 150 W frosted tungsten lamp. A stabilized ac power supply was used to obtain a light output stable to within ±0.5 percent. The turbidity was determined with the optical fibers placed in contact with the walls of the cuvettes. The incident light intensity at the cuvette was about 6.5 μW, while the transmitted light intensity ranged between 330 and 230 nW. The light intensity was measured with a Photodyne XL8 photometer/radiometer with sensor head model 250.

Results

The possible effects of mm-waves on yeast suspensions were monitored by measuring the growth rate of the cu

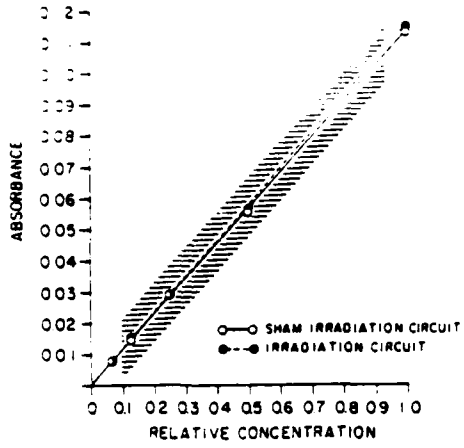

Fig. 5. A typical plot of absorbance versus concentration. A concentration of 1 corresponds to a 1:10 dilution of the overnight culture. The points represent the average of three measurements taken at the indicated concentrations. The slope for the "sham-irradiation" circuit had a coefficient of 0.112 with a 95 percent confidence limit of ±0.003. The "irradiation" circuit slope had a coefficient of 0.113 ± 0.002. The lines represent the fitted curves calculated with a linear regression program. The shaded bar indicates the absorbance/concentration range usually used for the experiments.

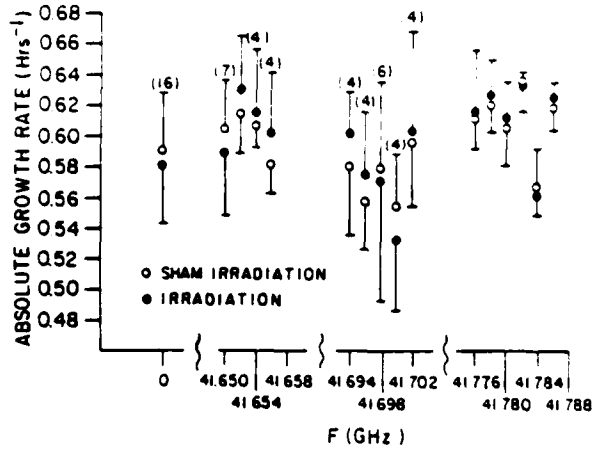

Fig. 6. Effects of millimeter-wave irradiation on the absolute growth rate of *Saccharomyces cerevisiae* as a function of frequency for an absorbed power of 20 ± 0.5 mW. Each point and bar represent the average ± 1 SD of the experiments at each frequency. Unless otherwise indicated in parentheses, three experiments were performed at each frequency.

tures and by performing a plate count at the end of each of the experiments. The absorbance was calculated as Abs = log (P_{in}/P_{tr}) where P_{in} was the light intensity transmitted through the reference cuvette filled with Sabouraud glucose broth and P_{tr} was the light intensity transmitted through the cuvettes filled with either the sham-irradiated or the irradiated suspension. Before each experiment, the light transmitted by the three cuvettes, all filled with the growth medium, was verified to be within the resolution of the photometer (i.e., 1 count = 1 nW).

The linearity of the response of the optical system of both circuits was determined before each experiment using a twofold dilution series, whose cell concentration range was close to that of the actual experiment. A typical absorbance versus concentration curve is shown in Fig. 5. For a total of 38 tests, the average percentage difference between the growth rates measured in the two circuits was +0.77 percent, with a standard deviation of 0.57 percent. The maximum difference ever detected was +2.5 percent.

The growth rate was obtained by using a regression analysis to fit the data to an exponential curve $C(t) = C_0 e^{\mu t}$, where $C(t)$ is the concentration at time t, C_0 is the initial concentration, and μ is the growth rate in h^{-1}. The percentage difference in the growth rate of yeast cells was calculated as $100 \times (\mu_i - \mu_s)/\mu_s$, where μ_i and μ_s are the growth rates for irradiated and sham-irradiated cultures, respectively.

We performed 16 experiments without either mm-wave irradiation or electrical heating, and under such conditions the average difference in growth rates between the "irradiated" and the "sham-irradiated" circuit was −1.5 ± 3.2 percent. The average 95 percent confidence limit of the estimated growth rate was about ±2.3 percent regardless of the circuit or of the presence of mm-waves.

From these data we can infer that, with at least three experiments for each frequency, the minimum difference L, significant at the 95 percent confidence limit, was about ±3.5 percent as calculated from $L = 1.96\ \sigma/\sqrt{n}$, where σ is the average standard deviation, 3.2 percent, and n is the number of experiments [26]. In the three frequency ranges that were covered, the selected frequencies were 2 MHz apart. At least three experiments were performed at each frequency; the actual numbers are indicated in Figs. 6 and 7.

In Fig. 6 the results of mm-wave irradiation on the growth rate of yeast measured optically are shown as averages of the absolute growth rates μ_i and μ_s. In Fig. 7(a) the same data are also plotted as a percentage difference of the growth rates. Paired t tests were performed on the absolute growth rates of irradiated versus sham-irradiated cultures, grouped by frequency. No statistically significant differences were detected ($P > 0.05$) at any of the selected frequencies.

For most of the experiments we also performed a plate count at the end of irradiation. The percentage differences between the number of viable population units of the irradiated culture minus those of the sham-irradiated are shown in Fig. 7(b). No statistically significant differences ($P > 0.05$) were detected by unpaired t test statistics performed for each experiment, except for some experiments at some of the frequencies at which $0.01 < P < 0.05$. This, however, never occurred for more than half of the total number of experiments done at each frequency.

We have also performed some experiments at higher power levels at a fixed frequency of 41.698 GHz. The results are showed in Fig. 8. No effects were detected even at these higher powers.

Discussion

In the past some investigators indicated that the mm-wave [1] at different frequency ranges alters the growth rates of *R. rubra* [1], *Sacch. cerevisiae* [2]-[6], *E. coli*

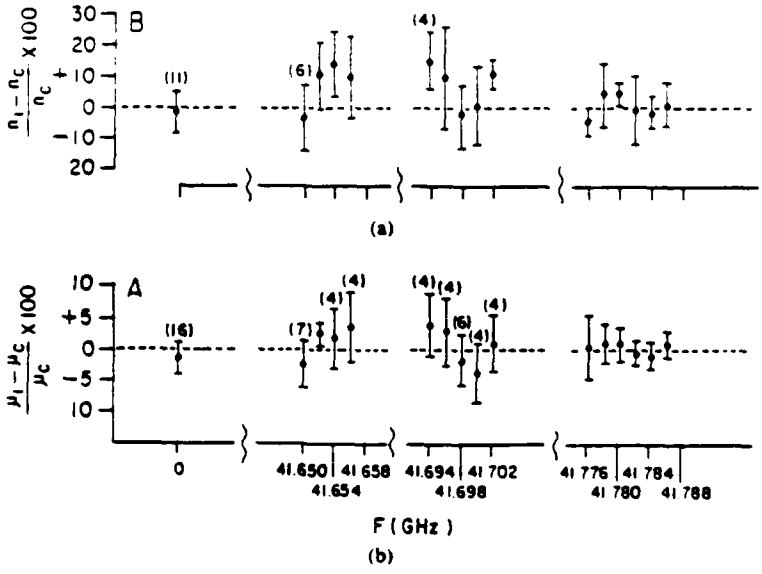

Fig. 7. Effects of millimeter-wave irradiation on *Saccharomyces cerevisiae* as a function of frequency. (a) Percentage differences of the growth rates. (b) Percentage difference in the number of viable populations units. Each point represents the average ±1 SD of the experiments at each frequency. Unless otherwise indicated in parentheses, three experiments were performed at each frequency.

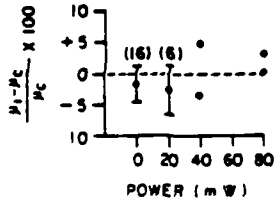

Fig. 8. Effects of higher power levels on the growth rate of yeast. Frequency fixed at 41.6980 GHz. The points at 40 and 80 mW represent the result of single experiments.

[7], and *C. albicans* [9]. Other reports also indicated that mm-waves enhance the induction of colicin [12] and of lambda phage [13] in *E. coli*. These reports prompted several comparative studies on prokaryotic and eukaryotic cells such as *E. coli* [16], *Sacch. cerevisiae* [17], BHK-21 [18], [19], and *S. typhimurium* [22], [23]. All these studies failed to indicate any nonthermal effect of mm-waves. In particular, a study on protein synthesis in mammalian cells BHK-21 exposed at various power levels failed to reveal any change due to mm-waves at 202 frequencies in the 38–48 GHz and 65–75 GHz ranges [19].

As shown in Figs. 6 and 7 the present study supports the conclusions that mm-waves do not affect in a detectable manner the growth rate or the viability of yeast cells. The average growth rate difference of irradiated cultures of *Sacch. cerevisiae* differed by no more than ±4 percent from the sham-irradiated at any of the selected frequencies, while the plate counts did not differ by more than ±15 percent. The larger differences in the plate count may have been due to slight initial concentration differences resulting from sampling errors that might have occurred when the circuits were loaded at the beginning of the experiment. This difference, however, would have not resulted in significant differences in the growth rates.

The spectral characteristics of our microwave source can be favorably compared to those of a similar experiment reported by Grundler *et al.* [6], where the bandwidth was ±0.5 MHz, the frequency stability was ±0.3 MHz, and the absolute frequency was accurate to within ±0.1 MHz. In particular, the use of very accurate frequency counters in both this study and the ones reported in [5], [6] excludes the possibility that we might have used absolute frequencies offest by more than a few hundreds of a kilohertz. Since a systematic scanning of even a small portion of the millimeter-wave band, 30–100 GHz, with a frequency step of 2 MHz would have required an inordinate amount of time, we have focused the experiments at the frequency ranges in which the largest effects have been reported in the past on the growth of *Sacch. cerevisiae* [2]–[6].

The objection could be made that the difference in the results could be ascribed to the differences in strain. This objection postulates the existence of a wild-type strain of yeast that is naturally sensitive to mm-wave radiation. On the basis of experience with induced sensitivity to physical agents, like UV for example, it seems extremely unlikely that this could happen. In the case of UV, for example, the resistance is obtained by selecting those cells (usually 1 in 10^6) that survive UV treatment. In our case the existence of a mm-wave sensitive strain would imply that either a) the strain used by Grundler and Keilmann has been used after becoming sensitive to exposure to mm-wave radiation, or b) we used a strain that for some reason had lost its sensitivity to mm-wave radiation. The probability of either phenomenon happening, even though the

exact probability cannot be determined, seems to be exceedingly small. Furthermore, biological effects of mm-waves have been reported in a variety of different living organisms.

It should be pointed out that the points and the bars shown by Grundler *et al.* in Fig. 9 of [5] do not represent the average and the standard error, respectively, of multiple experiments at the same frequency. Instead, the points are the results of single experiments and the "error bar" was obtained as follows. Two different spectrophotometers monitored the growth of two cultures prepared from the same suspension. The maximum percent difference, over several experiments without microwaves, between the growth rates of the two cultures was then used as the estimated error. Therefore, the "error bars" in Fig. 9 of [5] all duplicate the same information. This representation also implies that the authors of [5], [6] assume the estimated experimental error to remain constant regardless of the irradiation frequency. If all the points within ±4 percent, i.e., their estimated error, of Fig. 9 in [5] are considered as the experimental "noise," then the use of a three-point interpolated curve to connect all the experimental data with a resonant-like curve becomes quite unjustified. However, even though in [5], [6] there are not enough experimental data at most of the selected frequencies to claim a definite effect on the growth rate, there is an apparent similarity between the results obtained with two independent sets of experiments, using two different irradiation systems. Furthermore, this similarity is also supported by the cross correlation analysis of the two sets of experiments [5], [6].

The experiments reported in [2]–[6] were not done at constant mm-wave power; instead, the power varied from experiment to experiment between 5 and 40 mW, introducing thereby another uncontrolled variable in the experimental procedure. However, both in the present study and in the ones reported earlier [2]–[6], no cell was exposed continuously to mm-wave energy due to the small skin depth of mm-waves and either recirculation or stirring of the cell suspension. In fact, two of the more notable differences between the study in [2]–[6] and the present one are a) the average time for a single exposure (0.06 s versus 2.0 s for the present study); b) the average interval between subsequent exposures (1.2 s versus 30 s). Nevertheless, the total time that the average cell was exposed to mm-wave energy is comparable (5 percent versus 6.7 percent for the current study).

As a suggestion for future studies of biological effects of mm-waves, we would like to point out that in the past when the power used was less than 5 mW/cm^2 such effects have been considered "nonthermal" in nature [2]–[8], [13], [17], suggesting that the microwave-induced heating could not have caused the reported effect. However, the skin depth δ is very small in lossy materials such as biological media containing large amounts of water. For example in distilled water, δ is between 0.78 and 0.23 mm for frequencies in the range 30–300 GHz. This leads to very high values of the specific absorption rate (SAR) in the surface layers of the exposed medium even for apparently small incident power densities. For example, at 1 mW cm^{-2}, the surface SAR in water at 40 GHz is 18.4 W kg. On the other hand, the power density P decreases exponentially as $P = P_0 e^{-2x/\delta}$ where P_0 is the incident power density and x is the distance from the surface exposed to the incident field. Therefore, any organism located more than 3–4 skin depths from the exposed surface is subjected only to a negligible fraction of the incident mm-wave radiation.

It is therefore important to determine, on an individual basis, whether such high values of power deposition can lead to subtle thermal effects or not. Some of the reported effects have been more appropriately called nonthermal when either of the following circumstances occurred: a) the microwave irradiation induced an effect opposite to that expected by a comparable increase in temperature; b) when the effect appeared to be strongly dependent on the irradiation frequency.

Conclusions

The current experiments were specifically designed to gather data that would help establish the presence or absence of nonthermal effects and frequency specific effects of mm-waves on the growth of cells. This effect, first reported in 1968 [7] for the growth of *E. coli* was followed by other reports indicating specific induction of lysogenic *E. coli* [13], colicin induction [12], and others [1]–[6], [8], [9]. The fundamental and empirical significance of these alleged frequency specific actions of nonionizing radiation in the mm-wave range hardly needs any emphasis.

Contrary to the previous reports [2]–[6] from other investigators, millimeter waves did not induce any detectable effect on either the growth rate or the viability of yeast cells exposed for 4 h to ultrastable millimeter waves between 41.650 and 41.798 GHz. This report adds to a growing list of others [15]–[23] showing that under carefully controlled experimental conditions, no nonthermal effects of millimeter waves on unicellular organisms are evident.

This conclusion suggests extreme caution when unconfirmed reports are used as a basis for broad generalizations of biological effects of millimeter waves [24], [25] by authors who also suggest the possible use of mm-waves for cancer diagnosis [27], [28] and therapy [29], and a possible means of cell communication [24].

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Dr. Gandhi received the Distinguished Research Award of the University of Utah for 1979–1980 and a special award for "Outstanding Technical Achievement" from the Institute of Electrical and Electronics Engineers, Utah Section, in 1975. He edited a PROCEEDINGS OF THE IEEE Special Issue (January 1980) on Biological Effects and Medical Applications of Electromagnetic Energy. He is a past Chairman of the IEEE Committee on Man and Radiation (COMAR). His name is listed in [illegible].

APPENDIX B

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ABSENCE OF BIOLOGICALLY RELATED RAMAN LINES IN CULTURES OF BACILLUS MEGATERIUM

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Cultures of Bacillus megaterium have been examined with laser-Raman spectroscopy in the 20–300 and 800–1800 cm^{-1} ranges. No lines were detected in the 20–300 cm^{-1} range, and evidence is presented that sporadic lines observed in the other range can be ascribed to experimental artifacts.

The presence of time-varying lines in Raman spectra of cultures of Bacillus megaterium and Escherichia coli have been reported [1–3] in recent years. Webb claimed [3] that these lines could be observed only if the cultures were in an active metabolic state and had been "synchronized" with heat and cold shocks immediately before the recording of Raman spectra. Subsequently, several theoretical reports were published providing possible explanations [4–6] for the reported lines. These reports suggested either a relation between phonon condensation and cell cycle [4], or the presence of soliton vibrations in biomolecules [6], or just an empirical relationship among the reported lines [5]. However, a recent report [7] has questioned their presence due to the low concentration and very low Raman scattering cross section of the biomolecules possibly involved in the inelastic scattering process. Furthermore, in [7], experimental evidence was provided that, in general, an inhomogeneous bacterial culture might produce large fluctuations in Mie scattering during the first minutes after resuspension, and consequently altering the baseline of the recorded spectrum. Webb's reports, in particular, have been used by Fröhlich [8,9] and Webb himself [3] to support Fröhlich's hypothesis [9–11] concerning the possible presence of coherent excitations in biological systems. The existence of these coherent oscillations has been conjectured to result in frequency-dependent biological effects induced by low-power millimeter-wave exposure [9]; the presence of, or lack of, coherent oscillations has been also related to the cancer problem [8,12].

We report Raman experiments with cultures of B. megaterium which were prepared according to the experimental procedure reported by Webb in ref. [3]. The Raman spectra were recorded under flowing and nonflowing conditions either at room temperature (21–23°C) or at a constant temperature of 35°C. It is to be emphasized that in refs. [1–3] no mention is made about any kind of temperature control and that the spectra were recorded under nonflowing conditions.

Briefly, the B. megaterium cells were grown for 48 hours in nutrient broth at 32°C, washed three times in 0.9 percent NaCl solution, counted, and resuspended in saline at a concentration of 1×10^7 cell/ml. The cells were then placed in a water bath at 39°C for 15 min followed immediately afterwards by an ice bath for another 15 min. The suspension was then centrifuged and the cells resuspended at a concentration of 2×10^7 ml^{-1} in Davis Minimal Medium (DMM) (K_2HPO_4 7 g/ℓ, KH_2PO_4 3 g/ℓ, Na_3 citrate 0.5 g/ℓ $FeSO_4$ 0.01 g/ℓ, $(NH_4)_2SO_4$ 1 g/ℓ, $MgSO_4 + 7H_2O$ 0.1 g/ℓ, glucose 2 g/ℓ) diluted 1:3 in double distilled water at 35°C. All the suspending media were previously filtered through a 0.22 μ Millipore® filter. For each experimental session, a single bacterial suspension of about 10 ml was used and kept in a water bath at either 35°C or at room temperature. As a sample holder, we used a NSG Precision cell T73-FL that allowed us to circulate both the sample under investigation

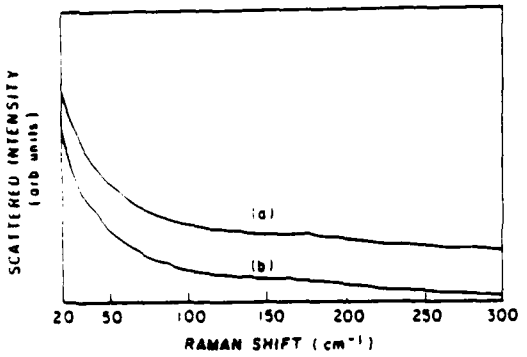

Fig. 1. Low-wavenumber Raman spectra of B. megaterium cell suspension after a heat and cold shock treatment and resuspention in nutrient medium. (a) Spectrum taken under flowing condition at 35°C. (b) Spectrum taken at room temperature under nonflowing conditions. The optical system employed a combination of a double monochromator plus a third monochromator with a spectral bandwidth of 4 cm^{-1} and with 1 s of integration time.

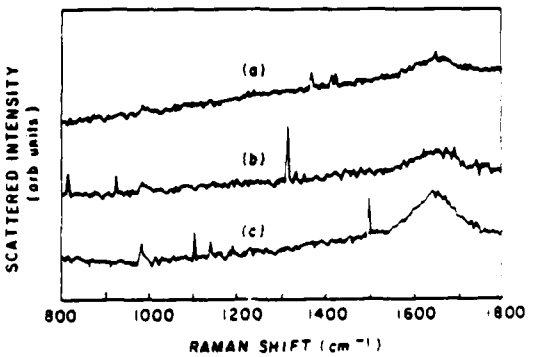

Fig. 2. Apparent Raman lines shown under flowing conditions in spectra of either (a) B. megaterium cells suspended in nutrient medium (DMM 1:3) at 35°C after heat and cold shock, (b) B. megaterium suspended in untrient medium after heat and cold shock at room temperature, or (c) suspension medium, Davis minimal medium 1:3. The optical system was the same as for spectra of fig. 1. The broad peak around 1640 cm^{-1} is due to the H–O bending mode of water, while the weak peak at 980 cm^{-1} can be ascribed to the presence of SO_4 group and K_2HPO_4 and KH_2PO_4 in the suspending medium.

and temperature-controlled water in an outer jacket. To establish sample flow, a peristaltic pump set at 5 ml/min was used.

The Raman system consisted of a Spectra Physics 164/09 Argon-ion laser operating at 4880 Å, a Spex plasma line filter, and a Spex third monochromator coupled to a Spex 1403 double monochromator. The detection system was composed of an RCA C31034 cooled GaAs photomultiplier and a Princeton Applied Research 1109 photon counter. The whole system was controlled by an LSI 11/23 minicomputer. The laser power at the sample was about 150 mW. Typically, spectra were recorded with a step size of 4 cm^{-1} and an integration time of 1 s.

For a total of over 15 spectra, no lines were ever detected in the 20–300 cm^{-1} region at any time between 10 min and 2 h 30 min after resuspension in DMM, at either room temperature or at 35°C and under flowing or nonflowing conditions. Typical spectra are presented in fig. 1. For over 50 spectra in the 800–1800 cm^{-1} range, range, we frequently observed random lines under flowing conditions from B. megaterium at 35°C or at room temperature and also from the suspension medium (DMM) alone, fig. 2. Only rarely did we observe small random lines from nonflowing preparations. Typical spectra under nonflowing conditions are presented in fig. 3. Both ranges were also examined in the anti-Stokes region and no lines at all were ever detected, in contrast to a previous report [3].

A possible explanation for these lines is the presence of either fluorescent particles or cell clumps passing randomly through the laser beam. As these random lines can be reproduced even in the absence of biological systems of any kind, we thus conclude that the observed Raman lines do not rise from active bacterial cultures. It is suggested that experiments be performed on simpler and more characterizable biological systems

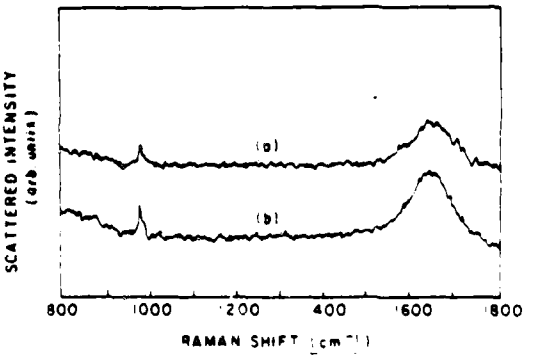

Fig. 3 Raman spectra taken under nonflowing condition. (a) B megaterium cell suspension. (b) Davis minimal medium.

such as selected antibody–antigene complexes and enzymatic reactions. With these systems, in fact, it would be possible to use greater concentrations and consequently to have a larger total scattering signal. Furthermore, the lines could be attributable, at least in principle, to specific biochemical species, thus allowing a more rigorous test of any hypothesis regarding possible presence of coherent excitations in biological systems.

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APPENDIX C

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ABSENCE OF LINES IN RAMAN SPECTRA OF LIVING CELLS

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A previous paper reporting the absence of biologically related lines in Raman spectra of Bacillus megaterium has been criticized. A reply to these criticisms is presented.

In the past few years some investigators have claimed the presence of lines in Raman spectra of metabolizing cells while no lines could be detected in spectra of resting cells [1–3]. Consequently other investigators have linked these alleged lines to internal vibrations of Davydov solitons [4,5], to coherent oscillations as postulated by Fröhlich [6] and to nonlinear-optics phenomena [7,8]. In our paper [9] we presented experimental data showing that no biologically related lines were found in Raman spectra of Bacillus megaterium suspensions under nonflowing conditions in the 20–300 and 800–1800 cm^{-1} regions and that under flowing conditions artifacts could be responsible for random lines appearing in the spectra of both cell suspensions and medium alone. For these experiments we closely followed the experimental protocol given by Webb in ref. [3].

Del Giudice et al. [10] have raised several questions about both the correctness of the conclusions and the validity of experimental data presented in our previous paper [9]. In particular our conclusions have been attributed more to an alleged "...preconceived philosophy..." and to "...motive behind the two recent reports..." rather than to a logical connection to the experimental data. We find these allegations out of place in a scientific forum, especially when these are totally unsubstantiated. After a very careful rereading of our paper we have not been able to find any suggestion of an alleged preconception. Furthermore Del Giudice et al. attributed to us a criticism of the work of Webb [1–3] that was, instead, pointed out by Cooper and Amer [11]. Cooper and Amer suggested that the Raman spectra of bacterial cells, prepared according to Webb [3] were likely to be featureless due to the relatively low cell concentrations.

In the present reply we hope to offer some clarifications and leave the conclusions to peers. Some of the questions asked in ref. [10] are, however, already explicitly answered in our original paper [9]. In the following, citations from our first paper [9] are in quotes:

(a) We did not use a nonnutrient medium. The legend of fig. 2 of ref. [9] contains a printing error: "untrient" instead of nutrient. We really wonder, however, how the word "untrient" can be read as nonnutrient.

(b) The experimental conditions of 35°C and room temperature, were used because the latter is the one used by Webb in ref. [3] and the former is an optimal growth temperature for B. megaterium.

(c) In addition to several experiments under nonflowing conditions, we used also a flow-through system, since this was also the recommendation of Drissler [12] to avoid excessive laser heating of the cells. We pointed out in our paper [9] that in the experiments reported by Webb [1–3] "...the spectra were recorded under nonflowing conditions".

(d) Del Giudice et al. [10] ask for clarification regarding the time elapsed between resuspension and recording of the spectra. In ref. [9] we clearly specified that "...no lines were ever detected in the 20–300 cm^{-1} region at any time between 10 min and 2 h 30 min after resuspension in DMM".

(e) All the apparent random Raman lines could be seen only in our spectra taken under flowing conditions. Such lines were narrow enough to be within one or two frequency steps (i.e. with a width of 4–8 cm^{-1}) of the computer driven spectrometer. This is compatible with the hypothesis that either air bubbles or microscopic fluorescent contaminants may have caused these, by randomly passing through the scattering volume.

(f) Under nonflowing conditions, the spectrum of the medium alone (Davis Minimal Medium 1 : 3 in H_2O) was completely reproducible, as were the featureless spectra of B. megaterium suspensions taken at any time after resuspension. It may be useful to point out that all the spectra of a single experimental session were taken using either the same suspension for an extended period of time (up to 45 min) or by changing the suspension every 10 min with another sample taken from a culture kept in a water bath at the selected temperature.

Furthermore, we have found that Kinoshita et al. [13] presented detailed calculations regarding possible artifacts that might have affected the intensity ratio of Stokes and anti-Stokes lines in Raman spectra of Chlorella pyrenoidosa [14,15]. These possible artifacts include the $1/\lambda^4$ dependence of the scattered light intensity, effective detection efficiency, resonance effect, laser-induced temperature increase and attenuation within the sample. These findings, along with our experimental data, show that several experimental artifacts can be introduced in the Raman spectroscopy of living cells and that to our knowledge no independent positive replication of the data presented in refs. [1–3,14,15] is available in the literature.

In conclusion we do not think that the alleged presence of lines in Raman spectra of baterial cells can be claimed to be "...non questionable..." [10] or that such "...revelations..." [10] can be taken as a scientific proof of coherent phenomena occurring in living cells as suggested by Webb [3], Fröhlich [6] and Del Giudice et al. [7,8,10].

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APPENDIX D

Raman spectroscopy of liposomes exposed to millimeter waves

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Several biological effects resulting from exposure to millimeter waves have been reported. In an attempt to determine if millimeter waves might affect the conformational state of membranes, sonicated liposomes have been irradiated with millimeter waves at 41.650 GHz, stabilized in frequency to $\pm$ 50 Hz. Raman spectra from the lipid vesicles were then collected in the conformational and in the C-H stretching region. No changes in either the Raman peak locations or relative intensities were detected upon millimeter-wave irradiation, either below or above the transition temperature of the phospholipid (41 °C).

Several investigators have suggested that millimeter waves (mm waves) affect a variety of biological functions including growth of bacterial cells,[1,2] DNA and RNA synthesis,[3] etc.[4,5] Unfortunately many of these reports lack essential details of the experimental procedure, thereby severely limiting the possibility of reproducing the experiments. An extensive review of the published literature is available.[6] Many times when duplication has been attempted, negative results have been reported. In particular, no effects were reported on cell morphology[7] and protein synthesis[8] in mammalian cells, BHK-21, and the growth rate of *E. coli*[9] and yeast[10] was not altered by exposure to mm waves. In 1968, with the appearance of the first experimental reports, Fröhlich proposed that mm waves may affect living systems by inducing coherent excitations in some parts of cells like DNA, cell membranes, and enzyme-substrate complexes.[4,11] Even though no specifics were given, two of the more interesting features of his model are its frequency dependence and an energy threshold for the onset of the effect. This interesting hypothesis, however, has not led to a prediction of specific frequencies or sites(s) of action for it. Nevertheless, based on the typical membrane thickness (100 Å) and on the speed of sound, Fröhlich has suggested that oscillations in the 10^{10}–10^{11} Hz range may be excited in cell membranes.[4]

Cytoplasmic membranes are an essential part of living cells. In addition to acting as the boundary between the internal and the external cell environment, membranes also perform essential functions such as selective ion pumping and cell recognition.[12] As depicted by the fluid mosaic model,[13] membranes are composed of lipids and proteins organized in a bilayer, 70–100 Å thick. Most of the functional

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properties of membranes arise from glycoproteins anchored to the bilayer itself.[14]

To study systematically the problem of identifying the specific site(s) of possible interaction of mm waves, we used Raman spectroscopy to determine if mm waves can induce conformational changes in sonicated vesicles exposed to 41 650 GHz, a frequency at which relatively large effects have been reported in the past on the growth rate of yeast.[2]

Phospholipids are characterized by a temperature T_c that marks the phase transition between gel ($T < T_c$) and liquid-crystalline phase ($T > T_c$).[15] In natural and artificial membranes (like liposomes), this transition temperature and the melting curve are known to be affected by several factors, including lipid moiety[16] and presence of proteins.[17]

Dipalmitoyl-phosphatidylcholine (DPPC) crystals (purity 99% —) were obtained from Sigma and used without further purification. For a typical experiment 0.8 mg of DPPC was mixed in 1 ml of buffer (Tris 10 mM, KCl 0.1 M, *p*H 7.2) and sonicated for 30 min, with the tube containing the suspension kept in an ice bath. The liposome suspension was used after filtration through an 0.8 μm Millipore^R filter. The predicted thickness of the liposomes is 70 Å,[12] and the average diameter is about 300 Å.[18]

Raman spectroscopy has been widely used in the past to determine the melting curves of phospholipids,[19] and the conformational changes induced in liposomes by various chemical and physical agents such as sonication,[20] cholesterol,[21] and proteins.[17] For the present study we used Raman spectroscopy to gather information on possible effects of mm waves on the conformation of phospholipids in liposomes above and below T_c.

The laser source was a Spectra Physics 164/09 argon-ion laser operating at 514.5 nm. A plasma line filter (Spex) was found to be essential to eliminate nonlasing plasma lines that would otherwise become apparent in the Raman spectra arising from the high scattering efficiency of the suspension. The power at the sample location was 400 mW. Spectra were collected with a Spex 1403 double monochromator coupled to a Spex third monochromator. The slit width was set at 400 μm, with a spectral bandwidth of 4 cm^{-1}. Spectra were collected at discrete wave numbers with an accumulation time of 5 s and a wave-number step size of 3 cm^{-1}. The Raman scattered light intensity was measured with an RCA C31034 cooled GaAs photomultiplier connected to a photon counter, Princeton Applied Research 1109. A custom-made microprocessor-based controller was used to control the scanning limits, integration time, and step size of the triple monochromator assembly.

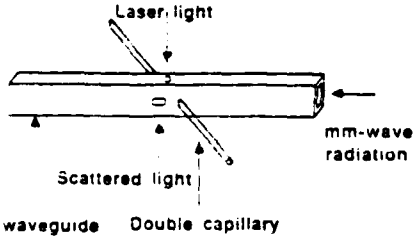

FIG. 1. Diagram of the waveguide capillary assembly.

The liposome suspension was placed in a borosilicate capillary, 0.2 mm i.d., that was then placed in a larger capillary, 0.65 mm i.d., in which temperature controlled water was circulated. The double capillary assembly was inserted, at a 45° angle, in a WR-19 waveguide. A diagram of the waveguide/capillary assembly is shown in Fig. 1. A thermocouple was placed inside the larger capillary to monitor the temperature of the water jacket. The maximum temperature increase caused by the laser exposure in the water jacket was about 2.5 °C.

The mm-wave source was a klystron, Varian 302-BT. The irradiation frequency was measured and phase-locked with an EIP 578 source-locking frequency counter, with a resulting short term stability (1 h) of $\pm$ 50 Hz and measured with up to 11 significant digits. The irradiation frequency was 41.650 GHz and was chosen on the basis of being a frequency at which a relatively large effect was reported previously on the growth rate of yeast.[2] The cw power in the waveguide was 30 $\pm$ 0.5 mW. The temperature increase of the water jacket induced by the mm-wave irradiation did not exceed 0.2 °C.

The power deposition pattern of the liposome suspension in the capillary was estimated numerically.[6] The calculations indicated that 11% of the incident power was absorbed in the sample, with a resulting average specific absorption rate of about 6000 W/kg. The power absorbed by the whole double capillary assembly was 80%. This was in fair agreement with experimental data.

Raman spectra were collected near the conformational frequency shift region, 700–1700 cm^{-1}, and C-H stretching region, 2800–3500 cm^{-1}. To study the possible effects of mm waves on liposomes both in the gel and in the liquid-crystalline phase, spectra were taken at 25 and 45 °C, i.e., below and above T_c, 41 °C, of DPPC.

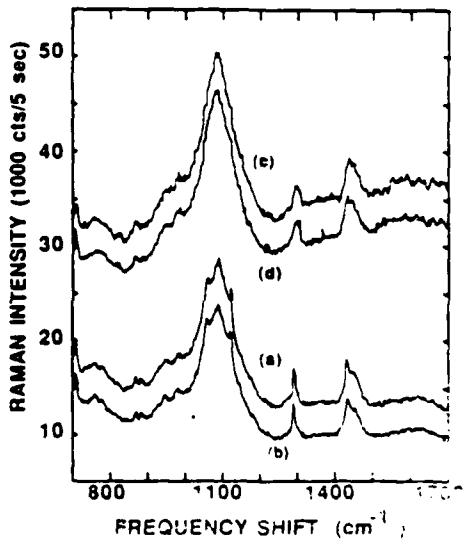

FIG. 2. Raman spectra of DPPC sonicated vesicles in the C-H stretching region with and without mm waves. Laser focused at a 5-mm beam diameter, time 5 s at discrete wave numbers spaced 3 cm^{-1} apart. The spectra have been plotted without any offset. The irradiation frequency was 41.650 GHz. (a) 25 °C nonirradiated, (b) 25 °C irradiated, (c) 45 °C nonirradiated, and (d) 45 °C irradiated.

Spectra collection was started within 1 h after sonication. The fluorescence background was partially quenched by exposing the sample to the laser beam for 30 min before starting the collection of spectra. Spectra at 25 °C were taken first without mm waves, and then with mm-wave irradiation. Millimeter-wave irradiation was interrupted before bringing the suspension to 45 °C.

The spectra taken with and without irradiation are shown in Figs. 2(a)–2(d) for the conformational region, and in Figs. 3(a)–3(d) for the C-H stretching region. The spectra were not smoothed. In agreement with previous reports,[22] the temperature-induced spectral changes are apparent from the changes in intensity of the lines of the skeletal optical modes at 1064 and 1128 cm^{-1} and in the CH_2 stretching modes at 2882 and 2845 cm^{-1}. On the other hand, no differences were apparent in either region, or at either temperature, between control and mm-wave irradiated samples.

Cell membranes have been suggested in the Fröhlich model as one of the possible sites(s) of interaction of mm waves with living systems.[4] Liposomes were used in the present study to provide a first step in identifying the possible site(s) of interaction of mm-wave irradiation using a relatively simple and well-characterized model of cell membranes. Raman spectroscopy was used not only for its capability in detecting conformational changes in phospholipids, but also because it allowed this determination to be performed simultaneously to the mm-wave irradiation. As Figs. 2 and 3 show, there was no evidence of effects induced by the mm-wave irradiation. This experiment, while it cannot confirm Fröhlich hypothesis, cannot prove it wrong either.

Even though liposomes, as prepared for the present study, cannot mimic the functional complexity of actual cell membranes, they offer the advantage of allowing accurate identification of the system being irradiated. For future experiments, however, liposomes could be prepared by incorporating integral proteins that, among other functions, will simulate the presence of ion pores,[23] a structure essential to membrane function. The incorporation of proteins, however, greatly alters the conformational state of phospholipids in vesicles prepared with pure lipids, while the protein structure is not altered when incorporated in a lipid bilayer.[17]

Furthermore, experiments at a lower frequency, 2.45 GHz, have indicated that microwaves increase the permeability of erythrocytes around T_c, but not at temperature different from T_c.[24] Raman spectroscopy could be used to determine if such changes in permeability can be ascribed to conformational changes in the lipid bilayer, when exposed either to 2.45 GHz or to mm-wave radiation.

Many effects have been ascribed to exposure to mm waves. Some investigators have even suggested the use of mm waves as a possible diagnostic and therapeutic tool for cancer.[4] The present study did not show any effect on the conformational state of model membranes exposed at 41.650 GHz, either below or above T_c. While this study cannot exclude the possibility that some biological functions might be affected in nonthermal ways be millimeter-wave irradiation, it adds to a growing list of reports showing the absence of statistically significant effect when a variety of living organisms are exposed to millimeter-wave irradiation.[6–10]

We want to thank David T. Borup for his help in calculating the power deposition pattern in the capillaries. This research has been sponsored by the U.S. Air Force, contract F33615-84-K-0613. L.F. also acknowledges the financial support of the Fulbright-Hays Commission for Cultural Exchanges Between Italy and the U.S.

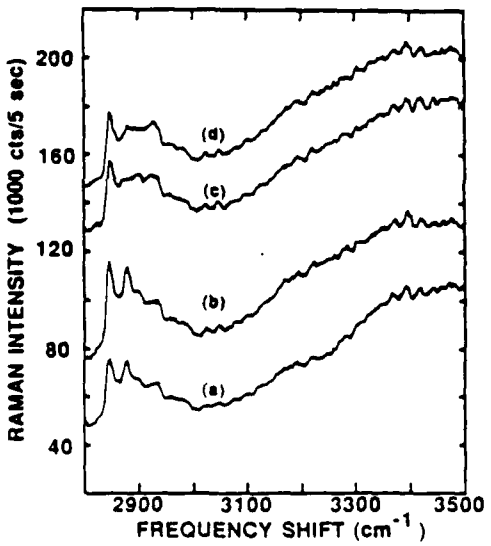

FIG. 3. Raman spectra of DPPC sonicated vesicles in the C-H stretching region with and without mm waves. (a) 25 °C nonirradiated; (b) 25 °C irradiated; (c) 45 °C nonirradiated; (d) 45 °C irradiated. The intensity scale is relative to spectrum (a). The other spectra have been plotted with the following offset: (b) 30 000 counts/5 s; (c) 60 000 counts/5 s; (d) 90 000 counts/5 s.

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APPENDIX E

MEASUREMENT OF COMPLEX PERMITTIVITIES OF BIOLOGICAL MATERIALS IN THE FREQUENCY BAND 26.5 - 60 GHz*,†

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ABSTRACT

Dielectric properties of biological materials have been measured in the frequency range of 26.5 to 60 GHz using a modified infinite sample method. In this method, impedance transformers are used to reduce the reflectivity of the infinite sample of biological material. The complex reflection coefficients were measured by an automated measurement system using three-probe reflectometer algorithm. An uncertainty analysis is performed to get an estimate of the errors in the measured complex permittivities. The experimental results and the Debye relaxation parameters are reported for ten percent NaCℓ solution, human blood, aqueous solution of bovine serum albumin and cell suspensions of saccaromyces cerevisiae.

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INTRODUCTION

Knowledge of the dielectric properties of biological substances is necessary for determining absorption of electromagnetic energy in biological bodies and for understanding biophysical mechanism of interaction with electromagnetic fields. Several investigators have reported sharp distinct resonances in the absorption of millimeter waves by various biological preparations [1-3]. Information on dielectric properties is useful to study these frequency-dependent effects and weak nonthermal interactions of biological systems with millimeter-wave radiation.

Previously, the dielectric properties of biological materials have been measured below 24 GHz by several investigators [4-6]. But, in the millimeter-wave range, the dielectric data have been sparse or nonexistent because of (1) limitations of conventional microwave techniques regarding small sample size associated with high attenuation, (2) requirements of precision measurement facilities and sophisticated instrumentation and (3) difficulties in eliminating experimental artifacts (e.g., air bubbles and nonuniform packing densities in cell suspensions, etc.). The dielectric properties of water have been measured at 34.86 and 70 GHz by Grant [7] and Szwarnowski and Sheppard [8], respectively. Also, Edrich and Hardy [9] have measured complex permittivities of muscle and fat tissues at frequencies from 40 to 90 GHz. For investigations of frequency-dependent absorption, the millimeter-wave absorption spectra of different biological substance, (e.g., DNA, RNA, etc.) have been measured by Gandhi, et al. [10].

Because of the ubiquitous presence of water in biological materials and its high attenuation (15-30 dB/mm) at millimeter wavelengths, a layer of less than 0.3 mm of biological substance absorbs 80 to 90 percent of the radiation. So, the Roberts and Von Hippel's shorted-line technique [11] and the microwave bridge technique [12] would require submillimeter sample lengths and complicated sample holders. Therefore, for accurate broadband measurements, it is undesirable to use these conventional microwave techniques. A review of other microwave measurement techniques for dielectric measurements of biological materials is given in [13, 14]. Attempts have been made to extend quasi-optical techniques towards the millimeter-wave region. At wavelengths above 5 mm, the quasi-optical technique (namely; dispersive Fourier transform spectroscopy) cannot be used because of weak radiative power from the mercury vapour lamp [15]. The other quasi-optical techniques (e.g., Mach-Zehnder spectrometer) are also not particularly suitable for biological samples at millimeter wavelengths.

The infinite sample method (sample thickness > 3-4 skin depths) has been used to measure the dielectric properties of low dielectric constant and high loss materials [16, 22]. This method obviates the need for complicated sample holders and submillimeter sample thicknesses because of the use of longer sample lengths. But, for high dielectric constant and high loss biological materials, this method is very inaccurate because of the need to measure high voltage standing wave ratios (VSWR) and small phase shifts (relative to short circuit). In the modified infinite sample (MIS) method, a quarter wavelength (or odd-multiples of quarter wavelengths) impedance transformer is used to

reduce the reflectivity of the infinite sample of biological material. With suitable choice of impedance transformers, this method gives accurate dielectric measurements for biological materials.

This paper describes MIS method for dielectric measurements of biological materials. For measurement of complex reflection coefficients, automated measurement systems have been developed which give reliable and reproducible data in the frequency range of 26.5 - 60 GHz. The three-probe reflectometer algorithm [20] has been used to improve the accuracy of reflection coefficient measurements. Uncertainties in complex permittivities and errors in the measured reflection coefficient have been discussed for the MIS method. Finally, the complex permittivity measurements were performed for various biological materials including 10 percent NaCl solution, human blood, aqueous solution of bovine serum albumin (BSA) and cell suspensions of saccaromyces cerevisiae. The complex permittivity data were analyzed using single relaxation Debye dispersion and Cole-Cole model.

THEORETICAL BASIS

Figure 1 shows the schematic diagram of the sample holder for MIS method. It is assumed that (1) only the dominant mode TE_{10} propagates through the filled sections of the rectangular waveguide, (2) the waveguide section containing the biological material is sufficiently long so that it can be considered as an infinite sample, (3) the biological material is homogeneous, and (4) the dielectric material for the impedance transformer is homogeneous and isotropic.

The characteristic impedance Z_{ci} normalized with respect to the characteristic impedance of the empty waveguide Z_o is given by [22]

$$Z_{ci} = Z_i/Z_o = \frac{\left[1 - (\lambda/\lambda c)^2\right]^{1/2}}{\left[\varepsilon_i - (\lambda/\lambda c)^2\right]^{1/2}} \qquad (1)$$

$$i = 1, 2$$

where $i = 1$ refers to the impedance transformer and $i = 2$ refers to the biological material, ε_i is the complex permittivity of the ith layer, $\lambda c = 2a$ is the cutoff wavelength of the empty waveguide and λ is the free-space wavelength.

The phase constant β_i is given by the relation

$$\beta_i = (2\pi/\lambda)\left[\varepsilon_i - (\lambda/\lambda c)^2\right]^{1/2} \quad i = 1, 2 \qquad (2)$$

The measured reflection coefficient ($\Gamma = |\Gamma| e^{j\theta}$) at $z = 0$ is related to the normalized input impedance by the following relation

$$Z_{in} = \frac{1 + \Gamma}{1 - \Gamma} \qquad (3)$$

using transmission line equations, the input impedance Z_{in} is given by

$$Z_{in} = Z_{c1}\left[\frac{Z_{c2} + j\, Z_{c1} \tan(\beta_1 \ell)}{Z_{c1} + j\, Z_{c2} \tan(\beta_1 \ell)}\right] \qquad (4)$$

By rearranging various terms, we get

$$Z_{c2} = Z_{c1}\left[\frac{Z_{in} - j\, Z_{c1} \tan(\beta_1 \ell)}{Z_{c1} - j\, Z_{in} \tan(\beta_1 \ell)}\right] \tag{5}$$

From Eq. 1, the complex permittivity of biological material is given by

$$\epsilon_2 = \left(\frac{\lambda}{\lambda c}\right)^2 + \frac{(1 - (\lambda/\lambda c)^2)}{Z_{c2}^2} \tag{6}$$

By substituting Eq. 1-5 in Eq. 6, one can obtain an explicit expression for the complex permittivity ϵ_2 of the biological material in terms of Γ, ϵ_1' (dielectric constant of impedance transformer) and ℓ (effective length of the impedance transformer). It is assumed that the dielectric materials for impedance transformers are low-loss materials (e.g., alumina). So, ϵ_1'' is negligible compared with ϵ_1'.

IMPEDANCE TRANSFORMERS AND SAMPLE HOLDERS

Figures 2 and 3 show the variation of $|\Gamma|$ and θ for the infinite sample of water with and without alumina impedance transformers. Some important observations can be made by inspecting Figs. 2 and 3. For a band of frequences close to f_{min}, the effects of introducing impedance transformers are (1) the increased variation in $|\Gamma|$ and θ and, (2) the reduction in $|\Gamma|$ from the values greater than 0.76 to less than 0.4. Because of these reasons, for a given precision in ϵ_2 of water, it is possible to allow higher measurement errors in $|\Gamma|$ and θ by using impedance transformers. This is shown in Table 1 for several frequencies by implementing a computer algorithm using Eq. 1 - 5. Since it is difficult to measure phase with an accuracy less than 0.5°,

particularly at millimeter wavelengths, the introduction of a transformer is advantageous.

Another advantage of using an impedance transformer is that it obviates the need for precision measurements of high VSWRS. Errors introduced in the measurements of high VSWRs are those due to the absence of strict square law response, appreciable rise and fall time for square wave source modulation and probe reflections due to excessive probe penetration [17-19]. Because of the limitations of millimeter wave instrumentation, many of these errors cannot be reduced significantly.

The impedance transformers may be quarter wavelength (or odd-multiples of quarter wavelength) waveguide sections of suitable solid material. In order to achieve the largest possible bandwidth (band of frequencies where $|\Gamma| < 0.4$), it is necessary to use a transformer of length one-quarter or three-quarter wavelength. For higher odd-multiples of quarter wavelengths there will be rapid variation of $|\Gamma|$ and θ which limits the useful bandwidth. It is required to select a material with dielectric constant between six and ten because of impedance-matching considerations. Alumina is chosen as the impedance transformer material because of its low-loss, good machinability and appropriate dielectric constant ($\epsilon_r = 9.25$). In order to cover the frequency range of 26.5 - 60 GHz, three alumina transformers of lengths 0.069, 0.163 and 0.126 cm were fabricated for frequency ranges 28-33 GHz, 42-45 GHz and 52-57 GHz, respectively. It should be noted that one-quarter wavelength transformer is used for 28-33 GHz frequency range, while three-quarter wavelength transformers are

used for 42-45 GHz and 52-57 GHz frequency ranges. Because of difficulties in fabrication of transformer thicknesses less than 0.05 cm, the three-quarter wavelength transformers were used for higher frequency ranges.

The complete sample holder consists of the impedance transformer and a section of the waveguide containing the biological sample. A plastic (Mylar™, 0.001" thick) window is used at $z = \ell$ interface (Fig. 1), so as to prevent the flow of biological sample into impedance transformer and slotted line. The dielectric discontinuity due to plastic window and discontinuities due to possible slight misalignment of flanges at the interfaces $z = \ell$ and $z = 0$ (Fig. 1) were taken into account by a small change (< 2 percent) in the length of impedance transformer. This is justified since small discontinuities can be modeled as susceptances which lengthen or shorten the transmission lines. The effective length of the impedance transformer is measured by using standard materials (e.g., water, saline, etc.) as the calibrating media.

THREE-PROBE REFLECTOMETER

In order to make precision measurement of $|\Gamma|$ and θ using an automated measurement system, the three-probe reflectometer formulation described by Caldecott [20] has been implemented. It uses standing wave (SW) voltage measurements for three equally spaced positions of the slotted line probe. It is assumed that the slotted line detector is in the square law region and the frequency is known accurately. Let V be the incident voltage and V_n is the SW voltage at the nth probe

position. Then

$$V_n = V \left[1 + |\Gamma| \, e^{j(\theta - \theta n)}\right] \tag{7}$$

$$P_n = |V_n|^2 = V^2 \left[1 + |\Gamma|^2 + 2|\Gamma| \cos (\theta - \theta_n)\right] \tag{8}$$

where θ_n is the phase shift corresponding to the distance to the load and back. There are three unknowns ($|\Gamma|$, θ and V^2) in Eq. 8. They are calculated from the three measured values of P_n. The complete formulation is given in [20]. The spacing between the neighboring probe positions is close to one-eighth of a guide wavelength ($\lambda g/8$) for maximum accuracy in $|\Gamma|$ and θ. The $|\Gamma|$ in this algorithm is inversely proportional to V^2, while θ is independent of V^2. So the errors in the calculated values of V^2 cause inaccuracies in $|\Gamma|$. For high reflection coefficient loads (e.g., short circuit, infinite sample of water, etc.) the errors in the calculated values of V^2 due to errors in the measured SW voltages are large. Hence, high values of $|\Gamma|$ cannot be measured accurately by this algorithm. Since reflection coefficient measurements for MIS method involve $|\Gamma| < 0.5$, this limitation is not important for our measurements.

MEASUREMENT SYSTEM

Two computer-controlled measurement systems covering the frequency ranges 26.5 - 40 GHz and 40 - 60 GHz have been developed for measurement of complex reflection coefficient. The measurement system (40 - 60 GHz)

is schematically illustrated in Fig. 4. The IMPATT oscillators have an amplitude and frequency accuracy of ± 0.06 dB and ± 3 MHz [10]. The ferrite modulator provides 1 KHz square wave modulation of the millimeter wave signal. The travelling probe of the slotted line was moved in steps of 0.3 mm by the computer-controlled stepping motor, so as to take 40 SW voltage readings of the standing wave pattern. Because of computer control of the stepping motor, an accuracy of ± 0.01 mm is realized for all probe positions. At each location of the probe, the SW voltage was measured by a tunable diode detector (sensitivity ≈ - 60 dBm) via SWR meter, A/D converter and HP-86B computer. The SWR meter was used as a high gain amplifier for the detected 1 KHz signal. The SW voltages are averaged over ten or more repetitive measurements (depending on the variance). Because of averaging, the precision of SW voltages is better than ± 0.05 dB.

The three-probe reflectometer algorithm [20] (which is implemented on HP-86B computer) is used to calculate more than 20 sets (depending on frequency) of $|\Gamma|$ and θ. The accuracy of the complex reflection coefficient ($\Gamma = |\Gamma| e^{j\theta_m}$) is improved by averaging the various values of $|\Gamma|$ and θ_m. At each frequency, it is necessary to ensure that the diode detector is in the square law region because of the changes in power output of the source and the detector response with frequency. This is done by adjusting the power level using the attenuator. The absolute phase θ (degrees) is obtained from the measured phase θ_m (degrees) by estimating the length ℓ between the starting probe position and the load. The following relationship is obtained from the transmission line theory.

$$\theta = \theta_m + 720\ \ell/\lambda g \qquad (9)$$

The length ℓ is calculated from short circuit phase measurement by using Eq. 9 for $\theta = 180°$. An accuracy of the order of 0.0016 λg is realized for length ℓ by frequency averaging of the length data and repetitive short circuit measurements. The measurements were not made in the frequency ranges 35 - 40 GHz and 46 - 50 GHz because of very low probe output from the slotted line and amplitude instability of the IMPATT oscillator. There were a few frequency ranges such as 33.5 - 34.5 GHz and 57.5 - 60 GHz where the measurements were not made because of frequency instability and low power output of the source.

UNCERTAINTY ANALYSIS

The total uncertainty in measurement of complex permittivity ($\varepsilon_2 = \varepsilon_2' - j\varepsilon_2''$) using the MIS method mainly depends on (1) errors in measured $|\Gamma|$ and θ, and (2) the errors in the measured dielectric constant and length of the impedance transformer.

The total uncertainties $\Delta\varepsilon_2'$ and $\Delta\varepsilon_2''$ are defined as [21]

$$\Delta\varepsilon_2' = \left[\left(\frac{\delta\varepsilon_2'}{\delta|\Gamma|}\ \Delta|\Gamma|\right)^2 + \left(\frac{\delta\varepsilon_2'}{\delta\theta}\ \Delta\theta\right)^2 + \left(\frac{\delta\varepsilon_2'}{\delta\varepsilon_1'}\ \Delta\varepsilon_1'\right)^2 + \left(\frac{\delta\varepsilon_2'}{\delta\ell}\ \Delta\ell\right)^2\right]^{1/2} \qquad (10$$

$$\Delta\varepsilon_2'' = \left[\left(\frac{\delta\varepsilon_2''}{\delta|\Gamma|}\ \Delta|\Gamma|\right)^2 + \left(\frac{\delta\varepsilon_2''}{\delta\theta}\ \Delta\theta\right)^2 + \left(\frac{\delta\varepsilon_2''}{\delta\varepsilon_1'}\ \Delta\varepsilon_1'\right)^2 + \left(\frac{\delta\varepsilon_2''}{\delta\ell}\ \Delta\ell\right)^2\right]^{1/2} \qquad (11)$$

where $\Delta\varepsilon_1'$ and $\Delta\ell$ are the errors in the dielectric constant and the effective length ℓ of the impedance transformer. $\Delta|\Gamma|$ and $\Delta\theta$ are the errors in the magnitude and phase of the reflection coefficient. The partial derivatives appearing in Eq. 10 and 11 cannot be evaluated analytically for the MIS method. So, a computer algorithm using Eq. 1 - 6 was used to calculate $\Delta\varepsilon_2'$ and $\Delta\varepsilon_2''$.

The primary sources of errors in $|\Gamma|$ and θ are the measurement errors in SW voltages and inaccuracies in the position of the slotted line probe. The other likely sources of errors are the reflections from the probe, amplitude and frequency drift of the IMPATT oscillators, square law response errors, appreciable rise and fall time of the square wave source modulation, etc. Based on our measurements with short circuit and standard materials (e.g., water), it is estimated that the errors in $|\Gamma|$ and θ are less than $\pm$ 1.5 percent and $\pm$ 1°, respectively. Table 2 shows the uncertainties of ε_2' and ε_2'' for $\pm$ 1.5 percent and $\pm$ 1° errors in $|\Gamma|$ and θ, respectively.

The dielectric constant of the impedance transformer material (Alumina) was measured at several frequencies (30 - 60 GHz) using the shorted line technique described by Roberts and Von Hippel [11]. The measured data give $\varepsilon_1' = 9.25 \pm 0.05$. This value is in fair agreement with those in the literature [23, 15] considering the differences in manufacturers, types of alumina and frequency ranges.

The effective lengths of impedance transformers are estimated by using water having a conductivity of 200 μS/m ($\sigma = 2 \times 10^{-4}$ S/m) as a standard material. Debye parameters for water at 23°C are assumed as $\varepsilon_s = 78.56$, $\varepsilon_\infty = 4.3$ and $\tau = 8.58$ ps [7], where ε_s, ε_∞ and τ are the low

frequency permittivity, high frequency permittivity and relaxation time, respectively. The physical lengths ℓ are varied (within ± 2 percent), so as to obtain minimum root-mean-square error (RMSE). The RMSE is defined as

$$RMSE = \left[\frac{1}{2N}\sum_{i=1}^{N} \left|\varepsilon_2\,(f_i,\ell) - \varepsilon^d\,(f_i)\right|^2\right]^{1/2} \qquad (12)$$

where $\varepsilon_2\,(f_i, \ell)$ is the measured complex permittivity calculated using $|\Gamma|$, θ, f_i, ε_1', and ℓ. f_i is the ith measurement frequency. $\varepsilon_2^d(f_i)$ is the complex permittivity calculated using single relaxation Debye dispersion model. N is the number of measurement frequencies. The $\varepsilon_2^d(f_i)$ is defined as

$$\varepsilon_2^d\,(f_i) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{(1 + j2\pi\, f_i \tau)} - \frac{j\sigma}{2\pi\, f_i \varepsilon_o} \qquad (13)$$

where ε_o is the free space permittivity. Figure 5 shows $\varepsilon_2\,(f_i, \ell_{min})$ and $\varepsilon_2^d(f_i)$ for various frequencies, where ℓ_{min} is the estimated effective length corresponding to minimum RMSE. The calculated RMSE of 0.78 for water gives an estimated accuracy of ± 2 μm for the effective lengths of impedance transformers. Table 3 shows the physical lengths and estimated effective lengths (ℓ_{min}) for impedance transformers.

Table 4 shows the uncertainties in ε_2' and ε_2'' because of ± 0.54 percent and ± 2 μm errors in the dielectric constant and the effective lengths of impedance transformers. The total uncertainties in ε_2' and ε_2'' calculated using Eq. 10 and 11 and Table 2 and 4 are less than ± 9.5 percent and ± 4.5 percent for the frequency range of 26.5 to 60

GHz. In general, the errors in the real and imaginary part of complex permittivities are less than these estimated uncertainties.

EXPERIMENTAL RESULTS AND DISCUSSION

In the absence of any frequency-dependent resonant effects, the dielectric properties of biological materials are characterized by a single relaxation Debye dispersion or the Cole-Cole equation because of the relaxation of water molecules. At millimeter wavelengths, the contributions due to other known relaxations (e.g., relaxation of the bound water, protein molecules, etc.) are negligible. The Cole-Cole equation is given by

$$\varepsilon_2 = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (j2\pi f\tau)^{(1-\alpha)}} - \frac{j\sigma}{2\pi f\varepsilon_o} \qquad (14)$$

while the single relaxation Debye dispersion is given by Eq. 13. The Cole-Cole parameter α indicates a spread of relaxation time centered about τ. The complex permittivity data are fitted to Cole-Cole equation and single relaxation Debye dispersion by the complex least-square formulation similar to the one described by Sheppard, et al. [24]. For all biological material used in our research, it is found that the RMSE is not significantly different for single Debye relaxation model ($\alpha = 0$) and Cole-Cole model. So, the single relaxation Debye dispersion model is adequate for the analysis of our complex permittivity data. All the measurements were made at the room temperature. But, the maximum change in temperature (from the mean temperature) during the experiments was limited to $\pm$ 1°C.

Figures 6-10 represent experimentally-measured and calculated (from Debye dispersion) complex permittivities for 10 percent NaCl solution, human blood, aqueous solution of BSA and cell suspensions of saccaromyces cerevisiae for two different concentrations. Table 5 shows the Debye parameters along with their standard deviations, mean temperatures and RMSE for various biological materials. For 10 percent NaCl solution, the ionic conductivity is calculated from Eq. 12 of [25]. The ionic conductivity for whole human blood is taken as 1.2 S/m from Cook's results [6]. It is assumed that these static values of conductivity are valid at high frequencies because of the negligible variation of conductivity with frequency for concentrated electrolytic solutions [4]. For all other biological materials, the ionic conductivity is taken as zero because the measured static conductivities (using conductivity meter) were less than 1 mS/m.

Figure 6 shows the measured and calculated complex permittivities for 10 percent NaCl solution. Stogryn [25] has given equations for calculations of Debye parameters using previously-published data. The ϵ_∞ value has been assumed to be 4.9. For 10 percent NaCl solution at 23°C ϵ_s, σ and τ calculated from Stogryn's equations are 52.2, 13.4 S/m and 7.5 ps, respectively.

Considering the differences in the ϵ_∞ value used by Stogryn and the value estimated from our data (See Table 5), the agreement in ϵ_s and τ is reasonable.

The measured complex permittivities of whole human blood are given in Fig. 7 along with the microwave frequency data obtained from Cook [6]. The Cook's data at 23°C were obtained by linear interpolation of

the data at 15, 25, and 35°C. The single relaxation Debye dispersion equation was fitted to our millimeter-wave data and Cook's microwave frequency data. These Debye parameters are given in Table 5. From [6], the Debye parameters for human blood at 25°C are

$$\varepsilon_s = 58.0,\ \varepsilon_\infty = 4.5,\ \tau = 9.0 \text{ ps and } \sigma = 1.2 \text{ S/m}.$$

Allowing for lower temperatures and possible differences in blood samples regarding cell concentration, these Debye parameters compare favorably with those given in Table 5. The blood sample was taken from a healthy male and the red blood cell concentration was between 4.8 to 5.0×10^6 per mm^3. 2.0 mg of heparin was added to the 10 mℓ sample so as to prevent coagulation. In addition to stirring the sample, the blood in the sample holder was replaced by the fresh blood (from the blood sample) quite frequently during the experiment. This reduces the effect of nonuniform cell packing density and heterogeneity. The same care was taken while using saccaromyces cerevisiae cell suspensions.

Figure 8 shows the measured and calculated complex permittivities for BSA solution. The solution was prepared at a concentration of 200 mg/mℓ (20 percent) by dissolving BSA in deionized water. The BSA used was supplied by CALBIOCHEM. The pH of the solution is measured to be 6.9. At microwave frequencies, the dielectric properties of 7 percent BSA solution have been reported by Buchanan, et al. [5]. But, no published data are available for 20 percent BSA solution at microwave and millimeter-wave frequencies. Because the protein content of many biological tissues is close to 20 percent, it is of interest to know the

dielectric behavior of 20 percent BSA solution. The Debye parameters obtained from the measured complex permittivities are given in Table 5.

The protein solutions at millimeter wave frequencies behave as a nonpolar solute (hydrated protein molecule) in a polar solvent (water). So, it is possible to calculate volume fraction (V_p) of the hydrated protein molecule by using an appropriate mixture equation. In the literature the Maxwell-Fricke mixture equation has been employed quite frequently for such purposes [26, 27]. So, we have used this mixture equation for calculating the volume fraction of the hydrated protein molecule. The Maxwell-Fricke equation is given by

$$\left[\frac{\varepsilon_m - \varepsilon_w}{\varepsilon_m + 2\varepsilon_w}\right] = V_p \left[\frac{\varepsilon_p - \varepsilon_w}{\varepsilon_p + 2\varepsilon_w}\right] \tag{15}$$

where ε_m, ε_w and ε_p are the relative permittivities of the mixture, water and hydrated protein molecule, respectively. This equation is strictly valid for suspensions of spherical particles. For non-spherical particles the factor 2 is replaced by a function of the shape of the particle. In case of BSA, the shape of the molecule is a prolate ellipsoid with an axial ratio of 3 [30]. The change in factor of 2 is small for BSA molecule because of the small variation of this factor for prolate ellipsoids of axial ratio less than 5 [31]. From Table 5, ε_m is 58.5 while the ε_p is assumed to be 5 [26]. ε_w is taken as 78.56 from [7]. So, $V_p = 0.206$. The bound water can be calculated from V_p by the following relation [28],

$$V_p = C\,(\bar{V} + \delta) \tag{16}$$

where C is the concentration of protein solution in gms per 100 mℓ and $\overline{V}$ is the partial specific volume which is 0.73 for BSA molecules [30]. From Eq. 16 the bound water is calculated as 0.302 g/g of protein. This is in good agreement with the value of 0.3 obtained by Buchanan, et al. and 0.42 obtained from Viscosity measurements [29].

Figures 9 and 10 give the measured and calculated complex permittivities for 10^7 and 10^9 cells/ mℓ saccaromyces cerevisiae cell suspensions. The cells used in this study were grown on sabouraud glucose agar (Neopetone^R 10 g/ℓ, glucose 40 g/ℓ, Baeto agar^R 15 g/ℓ) and stored at 4°C. From these cultures, organisms were inoculated in sabouraud glucose broth (Neopetrone^R 10 g/ℓ, glucose 20 g/ℓ, ph 5.6), incubated at 32°C on an orbital shaker (40 - 50 rpm) and centrifuged in the table top centrifuge. No published data are available on the dielectric properties of saccaromyces cerevisiae cells at microwave and millimeter-wave frequencies. The volume fraction of the cellular material and associated bound water can be calculated from Eq. 15 for 10^7 and 10^9 cells/mℓ suspensions. The permittivity (ε_p) is assumed to be 5. This assumption is justified because of the nonpolar nature of the cellular material and associated bound water at millimeter-wave frequencies. From Table 5, ε_m values for 10^7 and 10^9 cells/mℓ are 78.3 and 61.04. So, the volume fractions for 10^7 and 10^9 cells/mℓ samples are calculated (from Eq. 15) as 0.003 and 0.178, respectively.

CONCLUSIONS

A new method has been developed for measuring complex permittivities of biological materials at millimeter wavelengths. This

method employs an impedance transformer to reduce the reflectivity of the infinite sample of the biological material. Because of automated measurement system, good accuracy and reproducibility were observed in the measured complex reflection coefficient data. The experimental artifacts (e.g., air bubbles, nonuniform packing density, etc.) were minimized by the careful design of experimental procedures. From uncertainty analysis, it is estimated that the maximum errors in real and imaginary part of complex permittivities are less than 9.5 percent and 4.5 percent, respectively.

The results for 10 percent NaCℓ solution and whole human blood were compared with the previously published data. Good agreement was observed regarding Debye relaxation parameters between our data and the published data. The amount of bound water was calculated for the aqueous solution of BSA. It compares favorably with the value reported by other investigators. Finally, the dielectric measurements were performed on 10^7 cells/mℓ and 10^9 cells/mℓ saccaromyces cerevisiae suspensions. By using Maxwell-Fricke mixture equation, the volume fractions were calculated for these two concentrations. The complex permittivity data from all the biological samples can be explained on the basis of Debye relaxation of the water molecule and no frequency-dependent effects were observed.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help of Dr. Luciano Furia in preparing BSA and saccaromyces cerevisiae samples.

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Table 1. Maximum allowable errors in $|\Gamma|$ and θ for ± 10 percent precision in complex permittivity of water.

Frequency (GHz)	With impedance transformers		Without impedance transformers	
	$\left\|\frac{\Delta\|\Gamma\|}{\|\Gamma\|} \cdot 100\right\|$	$\Delta\theta$ degrees	$\left\|\frac{\Delta\|\Gamma\|}{\|\Gamma\|} \cdot 100\right\|$	$\Delta\theta$ degrees
28	4.1	2.7	0.8	0.35
30	8.7	3.7	0.9	0.38
32	7.7	3.1	1.0	0.41
42	4.5	2.9	1.0	0.35
44	6.0	2.0	1.1	0.37
52	3.4	3.8	1.4	0.42
54	7.5	3.0	1.4	0.43
56	8.1	2.4	1.5	0.44

Table 2. Uncertainties in ε_2' and ε_2'' for ± 1.5 percent and ± 1° errors in $|\Gamma|$ and θ, respectively.

Frequency (GHz)	$\left\lvert \frac{\Delta\varepsilon_2'}{\varepsilon_2'} \cdot 100 \right\rvert$	$\left\lvert \frac{\Delta\varepsilon_2''}{\varepsilon_2''} \cdot 100 \right\rvert$
28	2.4	2.1
30	2.6	1.6
32	2.4	1.5
42	2.8	1.9
44	3.9	1.9
52	4.5	2.8
54	2.3	1.3
56	3.7	1.6

Table 3. Physical and effective lengths of impedance transformers.

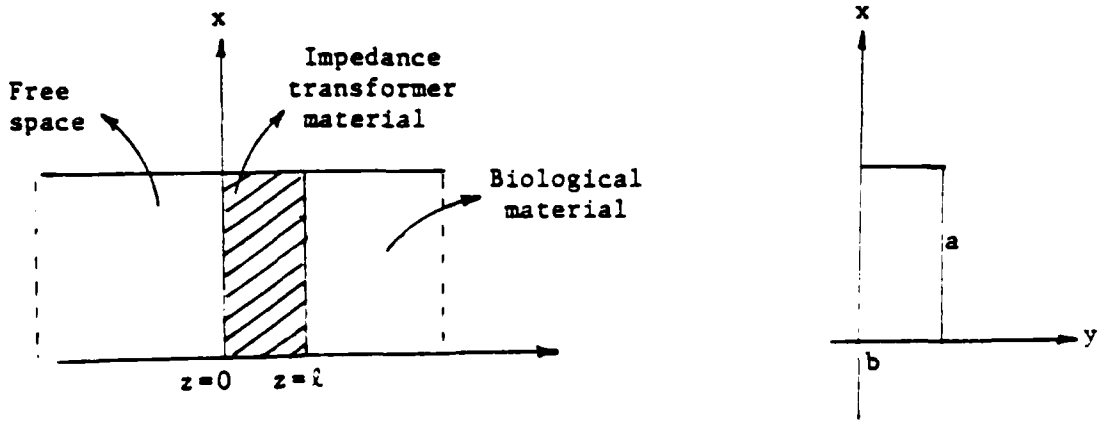
Frequency range of the impedance transformer (GHz)	Physical length (μm)	Effective length (μm)
28 - 33	686 ± 13	700 ± 2
42 - 45	1635 ± 13	1659 ± 2
52 - 57	1262 ± 13	1270 ± 2

Table 4. Uncertainties in ϵ_2' and ϵ_2'' for ± 0.54 percent and ± 2μm errors in ϵ_1' and ℓ, respectively.

Frequency (GHz)	$\left\lvert \frac{\Delta\epsilon_2'}{\epsilon_2'} \cdot 100 \right\rvert$	$\left\lvert \frac{\Delta\epsilon_2''}{\epsilon_2''} \cdot 100 \right\rvert$
28	2.5	1.2
30	2.8	1.3
32	3.2	1.3
42	7.9	2.3
44	8.6	2.6
52	6.5	2.3
54	6.9	2.5
56	7.3	2.7

Table 5. Debye parameters of biological materials.

Biological Material	Temperature °C	ε_s	τ	ε_∞	σ(S/m)	RMSE
10 percent NaCl solution	23	55.9±0.9	7.1±0.13	4.35±0.25	13.4	0.64
Human blood	23	58.0±1.6	9.64±0.24	4.24±0.24	1.2	0.91
RSA solution 200 mg/mℓ .	23	58.5±1.4	7.80±0.19	4.17±0.2	0.0	0.88
S. cerevisiae 10^7 cells/mℓ	24	78.3±2.0	9.0±0.3	4.00±0.3	0.0	1.10
S. cerevisiae 10^9 cells/mℓ	24	61.0±1.7	9.06±0.29	4.90±0.3	0.0	0.79



Layer number	0	1	2
Characteristic impedance	Z_o	Z_1	Z_2
Complex permittivity	1	ε_1	ε_2

Fig. 1. Waveguide sample holder for the MIS method.

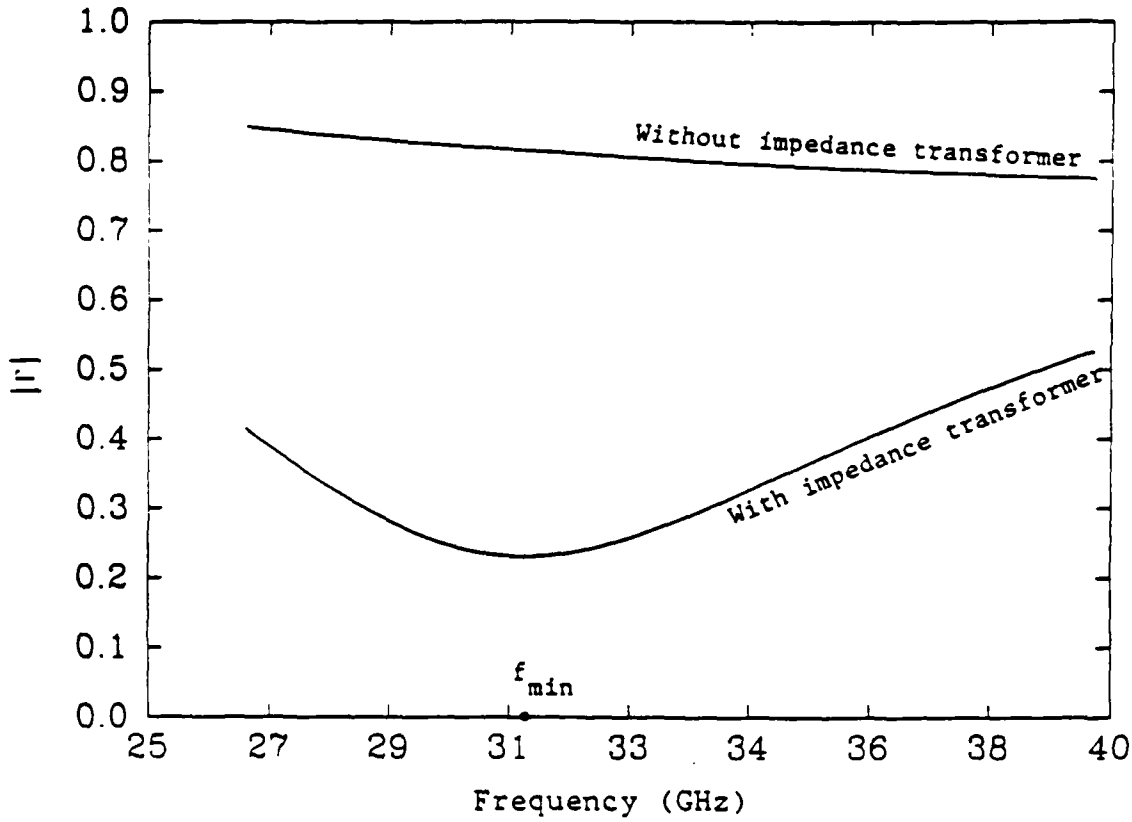


Fig. 2. Calculated variation of $|\Gamma|$ with and without impedance transformer for an infinite sample of water.

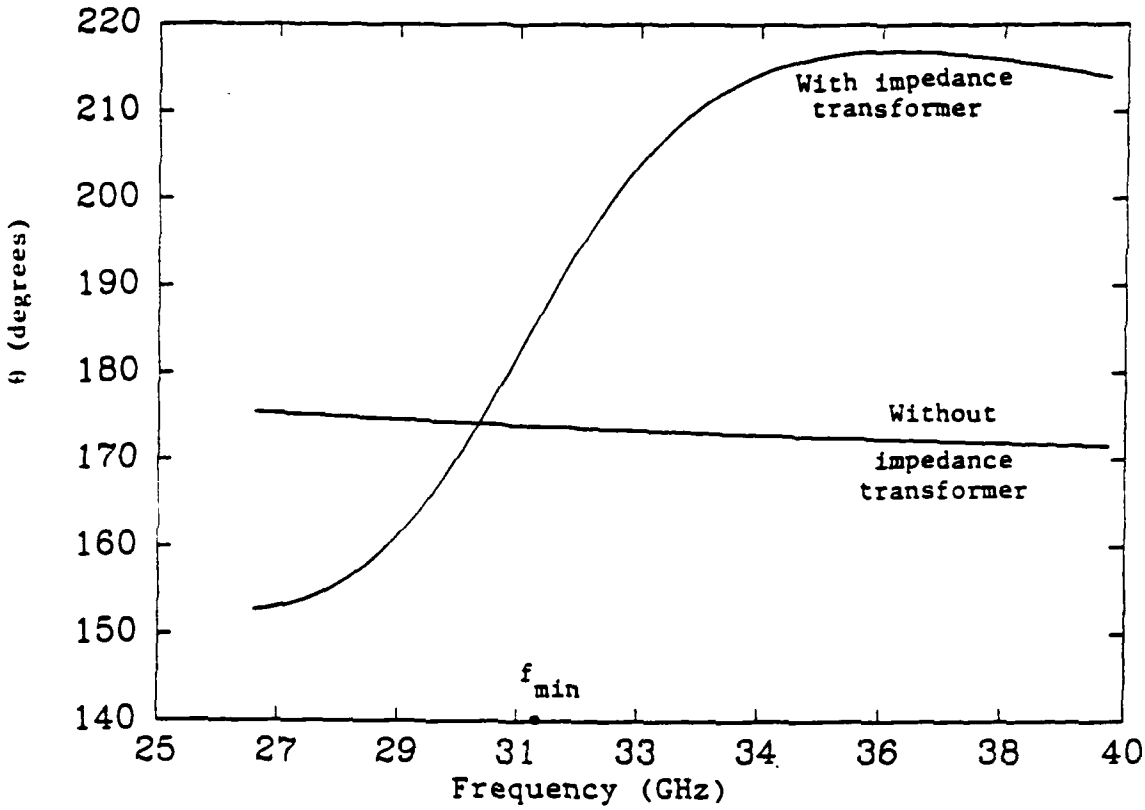


Fig. 3. Calculated variation of θ with and without impedance transformer for an infinite sample of water.

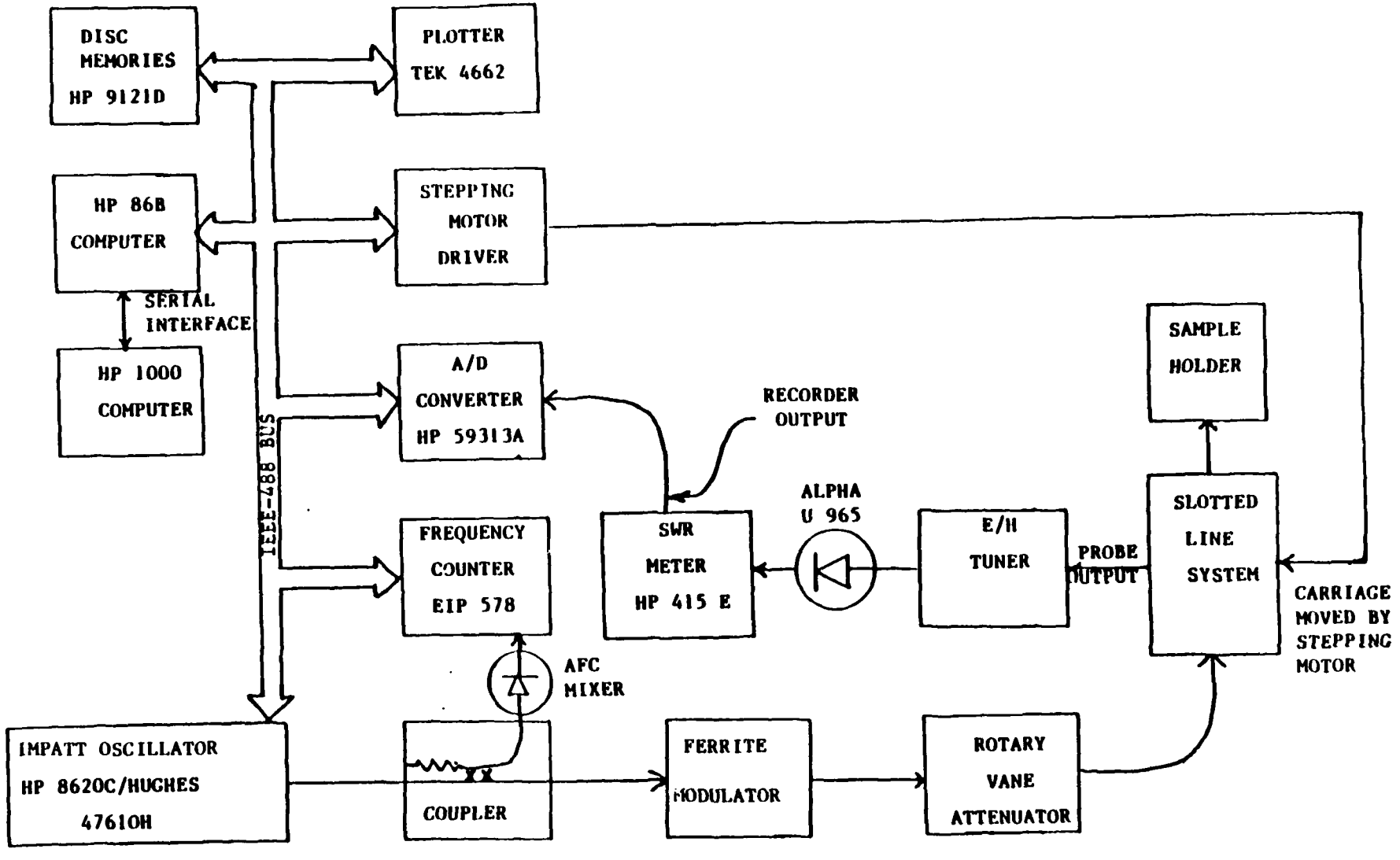


Fig. 4. Block diagram of automated measurement system.

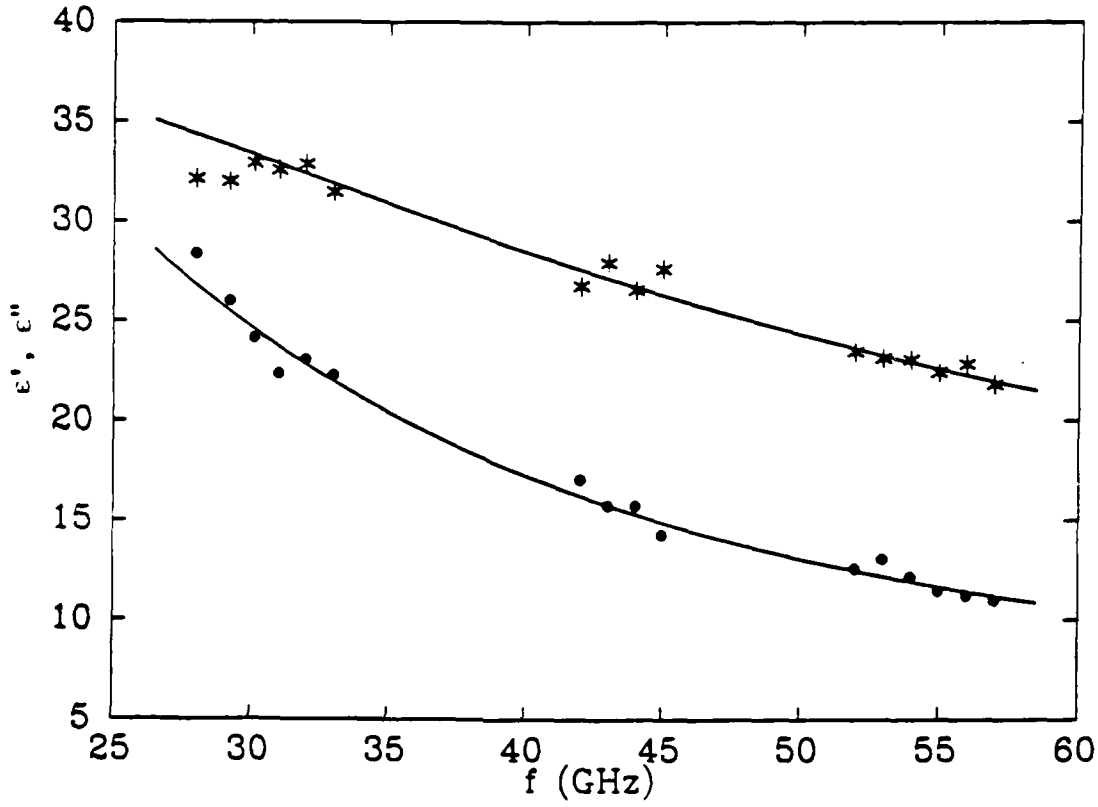


Fig. 5. Measured complex permittivities (• real part, * imaginary part) and calculated complex permittivities (solid line) for water at 23°C.

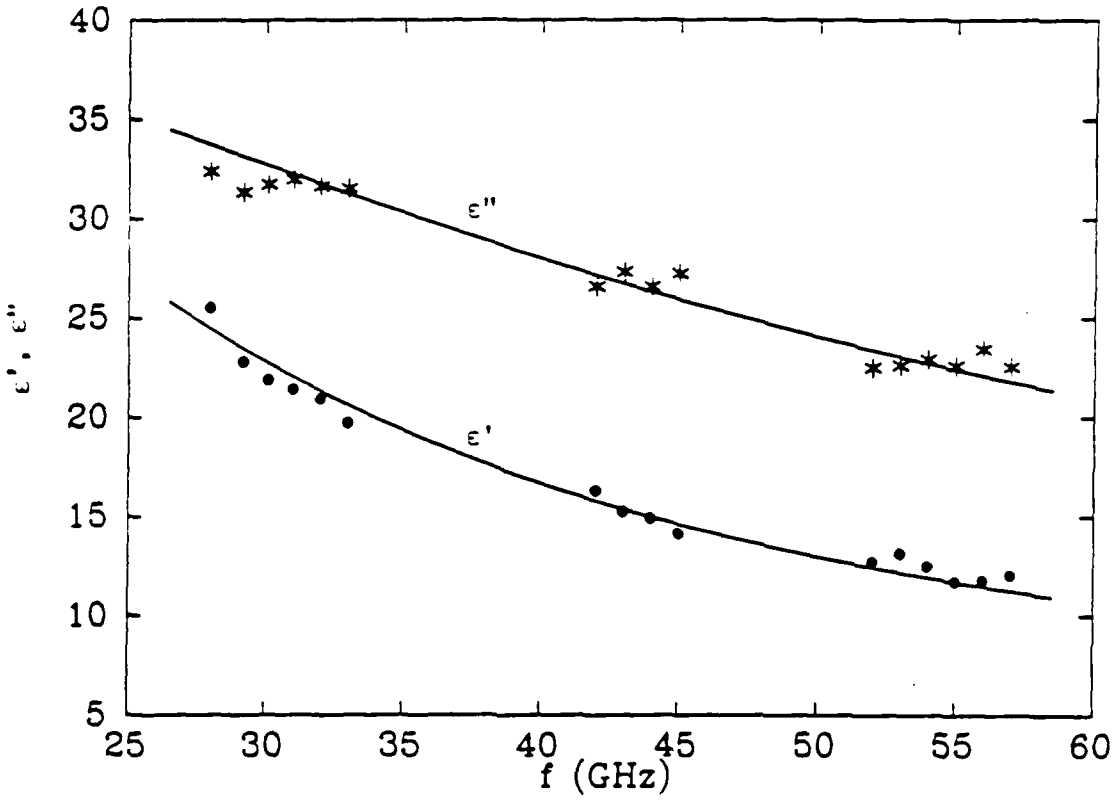


Fig. 6. Measured complex permittivities (• real, * imaginary) and complex permittivities (solid line) calculated from Debye dispersion for 10 percent NaCℓ solution.

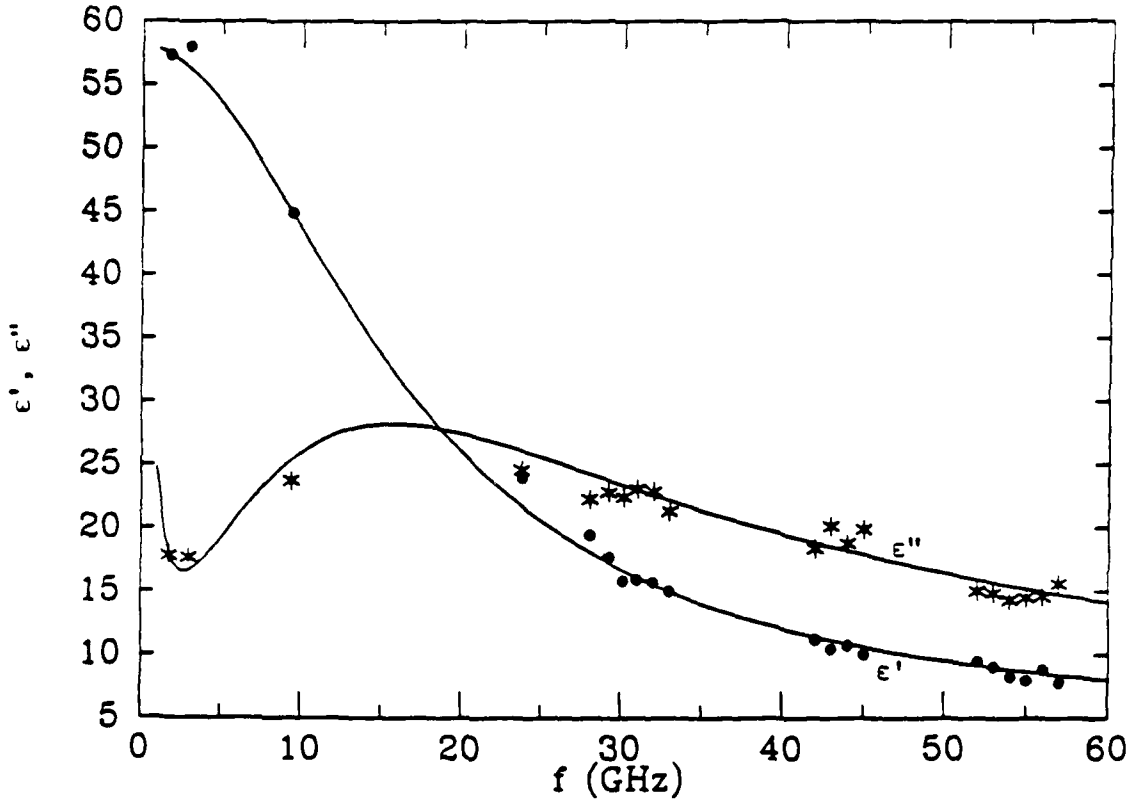


Fig. 7. Measured complex permittivities (• real, * imaginary) and complex permittivities (solid line) calculated from Debye dispersion for human blood. Results below 25 GHz are taken from [6].

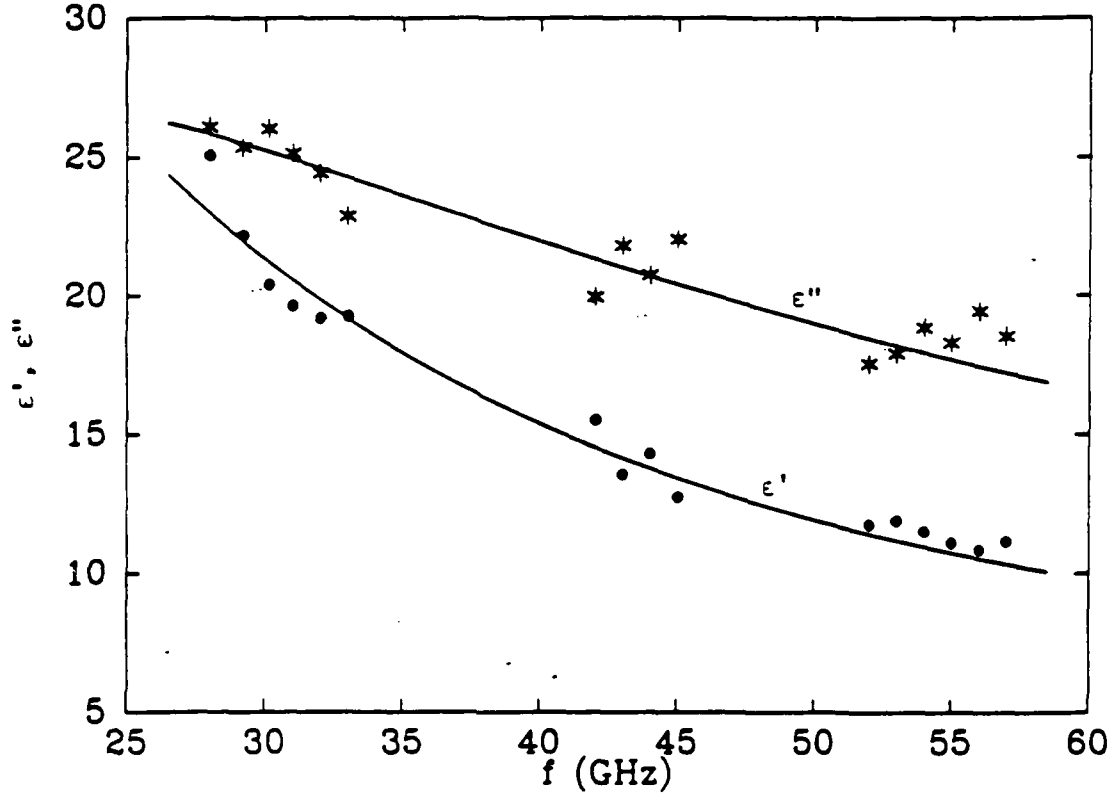


Fig. 8. Measured complex permittivities (• real, * imaginary) and complex permittivities (solid line) calculated from Debye dispersion for bovine serum albumin solution.

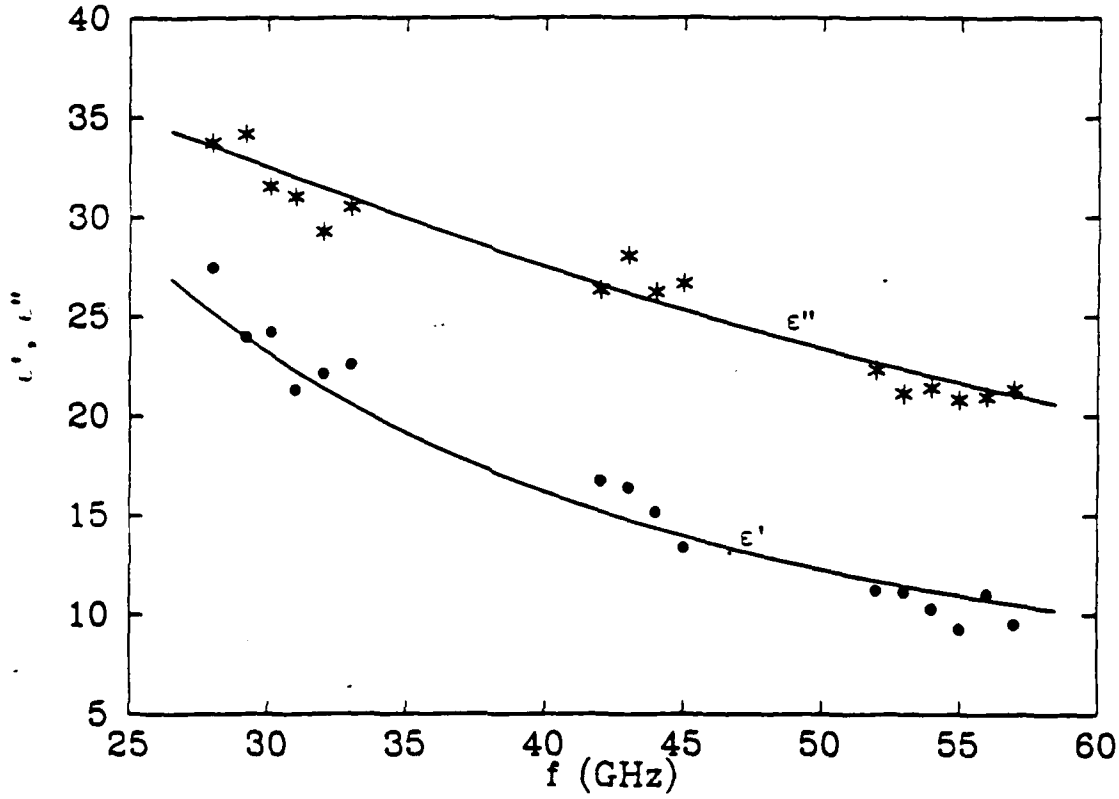


Fig. 9. Measured complex permittivities (• real, * imaginary) and complex permittivities (solid line) calculated from Debye dispersion for 10^7 cells/ mℓ saccaromyces cerevisiae sample.

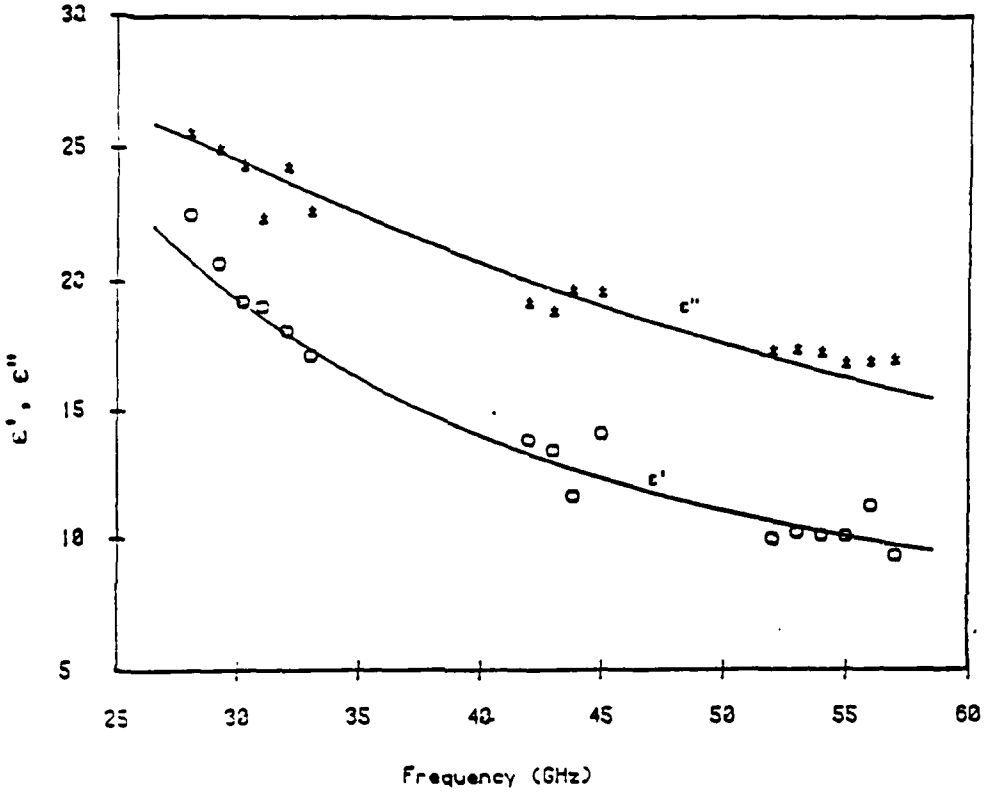


Fig. 10. Measured complex permittivities (o real, * imaginary) and complex permittivities (solid line) calculated from Debye dispersion for 10^9 cells/mℓ saccaromyces cerevisiae sample.

APPENDIX F

COMPLEX PERMITTIVITY OF THE HUMAN SKIN IN VIVO AT MILLIMETER WAVELENGTHS*,†

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ABSTRACT

A computer-controlled measurement system has been developed for complex permittivity measurements of human skin in vivo at millimeter wavelengths. An area of the human body is pressed against the waveguide flange for measurement of complex reflection coefficient. A quarter wavelength impedance transformer is used to reduce the reflectivity of the human skin. The complex permittivity of the human skin in vivo was calculated from the measured complex reflection coefficient by using a variational formulation involving multimode analysis. A single relaxation Debye dispersion is fitted to our millimeter-wave data and the microwave frequency data reported by other investigators. The complex permittivity values for human skin in vivo were much smaller than those reported for excised human skin tissue used in the past. The difference in complex permittivity values between excised skin tissue and skin tissue in vivo may be due to higher solid content of the skin in vivo.

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†Preprint of article submitted to IEEE Transactions on Biomedical Engineering.

INTRODUCTION

With the increasing use of millimeter-wave radiation for communications and radar, it is necessary to know the dielectric properties of various human tissues for evaluation of potential hazards to humans. At millimeter-wave frequencies, the absorption of electromagnetic radiation is mostly restricted to the skin because of the submillimeter depths of penetration [1]. So, the knowledge of dielectric properties of human skin in vivo is of prime importance for quantifying hazardous effects of electromagnetic waves in the millimeter-wave range. Also, for the study of millimeter-wave thermography, it is useful to know the dielectric properties of live human skin. In the past, the dielectric properties of excised human skin tissue have been measured below 30 GHz by Cook [2], England [3,4] and Schwan [5, 6]. Since the dielectric properties of biological tissues above 100 MHz are determined by their water, salt and protein content [7], the use of dielectric properties of excised skin samples to characterize live skin is justified only if there is no difference between excised and live tissue materials regarding their water, salt and protein content. Hence, the variation of blood content between live and excised skin tissue can cause significant changes in the complex permittivities. Because of these reasons, it is desirable to measure skin dielectric properties under conditions in vivo.

Recently, Hey-Shipton, et al. [8] have reported complex permittivities of human skin in vivo over the frequency range of 8 to 18 GHz. But their complex permittivity values are inaccurate below 11 GHz

because of reflections from the back of the skin at lower frequencies. We have previously presented complex permittivity measurements of human skin *in vivo* over the frequency range of 28 to 33 GHz [9]. In this paper, we are reporting complex permittivity data for human skin *in vivo* (palm region) between 28-57 GHz. The measurements were made on a newly-developed computer-controlled measurement system which measures the magnitude and phase of the reflection coefficient. The complex permittivities of the skin are obtained from measured reflection coefficients by using an algorithm which is an extension of multimode analysis given by Galejs [10].

The Debye relaxation model is fitted in the least-square sense on our millimeter-wave data and microwave frequency data from Hey-Shipton, et al. [8]. The emissivity of the human body and the attenuation coefficient of the human skin are calculated over the frequency range of 10 to 60 GHz using the complex permittivity values obtained from the Debye relaxation model. The complex permittivity values of human skin *in vivo* given by the Debye relaxation model fitted to our data and Hey-Shipton's microwave data are considerably lower than those given by Johnson and Guy [11] and Cook [2]. For example, at 2.45 GHz the complex permittivity value obtained from Johnson and Guy [11] is (47 - j16.2) for excised skin tissue while the complex permittivity value given by our Debye relaxation model for human skin *in vivo* is (25.0 - j13.6). The difference in the complex permittivity values between excised skin tissue and human skin *in vivo* may be due to higher solid content (proteins and lipids) [7], approximately 53.12 percent for skin tissue *in vivo* compared to 36.56 percent for the excised skin tissue.

MEASUREMENT SYSTEM

A solid-state, computer-controlled measurement system operating at frequencies from 26.5 to 60 GHz has been developed for measurement of magnitude and phase of the reflection coefficient. The measurement system is schematically illustrated in Fig. 1. The key components of the measurement system are the precision slotted line, the diode detector for standing wave measurements, frequency counter and HP 86B computer for overall automation. The traveling probe of the slotted line is moved in steps of 0.3 mm by the computer-controlled stepping motor. A large dial indicator calibrated in 0.01 mm increments gives the position of the probe. At each location of the probe, the standing wave voltage is measured by the diode detector (sensitivity = - 60 dBm) via SWR meter, A/D converter and HP-86B computer. By averaging 10 or more repetitive measurements of standing wave voltages, the extreme variation was limited to ± 0.05 dB. The standing wave voltages were measured at 40 equally spaced positions of the slotted line probe. The phase and the magnitude of the reflection coefficients are obtained by implementing a three-probe reflectometer algorithm given by Caldecott [12]. For a given setting, between 20 to 35 independent values of the phase and magnitude of the reflection coefficient are obtained due to different combinations of the standing wave voltages. The accuracy of the reflection coefficient measurements is improved by a factor of 5 - 6 upon averaging these reflection coefficient values. The absolute phase ϕ (degrees) of the reflection coefficient is obtained from the measured phase ϕ_m (degrees) of the human skin and the estimated length

ℓ between the starting probe position and the load by using the following equation:

$$\phi = \phi_m + 720\ell/\lambda g \qquad (1)$$

The length ℓ is estimated from short circuit phase measurements while λg, the guide wavelength is calculated from frequency measurements.

SAMPLE HOLDER FOR IN VIVO MEASUREMENTS

Figure 2 gives an arrangement for measurements of the human skin in vivo. An area of the human body is pressed against the waveguide flange for measurement of reflection coefficient. A quarter wavelength impedance transformer of Teflon™ is used to reduce the reflectivity of the human skin. The palm region of the human body is chosen for measurements because the skin is thick in this area (3 mm) and has a relatively smooth surface.

CALCULATION OF COMPLEX PERMITTIVITIES

The flow chart for calculation of complex permittivities of human skin in vivo from the measured complex reflection coefficient is given in Fig. 3. The key parts of this flow chart are the calculation of input admittance, admittance transformation for Teflon™ impedance transformer and calculation of new ε^* guess by zero finding technique.

It is assumed that human skin is nonmagnetic and its inhomogeneities are small compared with the wavelength of the electromagnetic waves. In order to model live human skin as semi-infinite half space,

it is necessary to assume that skin is lossy enough that the reflections from the back of the skin layer can be neglected. In practice, these assumptions are reasonable above 11 GHz. The input admittance due to human skin in vivo can be calculated by solving the radiation problem of a rectangular waveguide with the semi-infinite flange terminated by a semi-infinite half space of lossy dielectrics. We have implemented a formulation given by Galejs [10] for stratified plasma for calculation of input admittance. This formulation gives the input admittance of a smaller waveguide radiating into a bigger waveguide containing the biological material. For lossy dielectrics (e.g., human skin in vivo) it has been found that the semi-infinite half-space is simulated by the bigger waveguide if the ratio of bigger to smaller waveguide is greater than 30. The number of propagating and nonpropagating TE and TM modes in bigger and smaller waveguides is increased until the convergence is obtained in input admittance values. It has been found that the converged values of input admittance are obtained if all possible values of m and n (for TE_{mn} and TM_{mn} modes) are taken for $0 \leq m \leq 250$ and $0 \leq n \leq 250$.

The admittance transformation due to Teflon™ impedance transformer is calculated using transmission line equations. The implementation of zero finding technique involved prediction of a new guess for ϵ^* which corresponds to a zero of error function s. The Muller Method with Deflation [13] is used for calculation of zeros of error function s.

In order to test our formulation for calculation of input admittance, we have compared our results for the case shown in Fig. 4 to those obtained from the Waveguide Handbook [14]. The input admittance

(0.5 + j0.167) calculated using our program compares favorably with the input admittance (0.5 + j0.17) given by the waveguide handbook.

A flow chart similar to Fig. 3 has been implemented by Decreton and Gardiol [15] for nondestructive testing of dielectric materials. From Fig. 4 of [15], the calculated complex permittivity value is (7.2 - j5.9) for the following case:

VSWR = 4.0, Phase Shift = -4°

Frequency = 10 GHz, WR - 90 waveguide

The schematic diagram for this case is similar to Fig. 2. In the Decreton and Gardiol [15] method, however, no impedance transformer was used.

For this case, our method gives the complex permittivity value of (7.6 - j5.6). There is a close agreement between these two complex permittivity values. So, it provides a good test case for the accuracy of our in vivo computer algorithm. The standard materials (materials of known dielectric properties) with complex permittivity in the range of human skin in vivo are not available for frequency band 26.5 - 60 GHz. So, the in vivo computer algorithm has not been tested by measuring complex permittivities of standard materials.

EXPERIMENTAL RESULTS

In order to calculate complex permittivities using the flow chart given in Fig. 3, it is necessary to know the dielectric constant and the length of the impedance transformers. At millimeter wavelengths, the

dielectric constant of Teflon™ has been found to be 2.05 with an accuracy of ± 1 percent [16, 17]. The lengths of the impedance transformers were measured with an estimated accuracy of ± 5 μm from electrical length measurements. The shorted line technique described by Roberts and Von Hippel [18] was used for electrical length measurements.

Using the measurement system illustrated in Fig. 1, the magnitude and the phase of the reflection coefficients were measured for the arrangement shown in Fig. 2. The frequency ranges 28 - 33 GHz and 42 - 57 GHz are covered by using two quarter wavelength transformers. The measurements were not performed in the frequency ranges 35 - 40 GHz and 46 - 50 GHz because of the low probe output from the slotted line and power instability of the IMPATT oscillator, respectively. Based on our measurements with standard materials (e.g., water) used for modified infinite sample method [19], it is estimated that the errors in the magnitude and the phase of the reflection coefficients are less than ± 1.5 percent and ± 1°, respectively. The uncertainties in the calculated complex permittivities ($\varepsilon^* = \varepsilon' - j\varepsilon''$) are mainly due to: 1. The errors in measured reflection coefficient ($\Gamma = |\Gamma| e^{j\theta}$), and 2. The errors in the dielectric constant (ε_r) and the length (ℓ) of the impedance transformer. The procedure for calculation of uncertainties in the complex permittivities is similar to the one described in [19, 20]. The maximum uncertainty in ε' and ε'' of the human skin <u>in vivo</u> due to measurement errors in $|\Gamma|$, θ, ε_r and ℓ is less than 14 percent and 6 percent, respectively. The choice of the palm region of the human body for complex permittivity measurements minimizes errors due to improper skin contact.

Figure 5 shows the measured complex permittivity (• for real part and * for imaginary part) of the first author's skin at millimeter wavelengths. The microwave frequency data from Hey-Shipton, et al. [8] are also included in Fig. 5. The complex permittivity data were fitted to a single relaxation Debye dispersion; i.e.,

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + j2\pi f\tau} - \frac{j\sigma}{2\pi f\,\varepsilon_o} \qquad (2)$$

where ε_s and ε_∞ are the low- and high-frequency limits of the permittivity, τ is the relaxation time, f is the frequency and σ is the ionic conductivity. For human skin in vivo, σ is assumed to be 1.4 S/m from Cook's [2] results for excised human skin tissue. The Debye parameters (ε_s, ε_∞, and τ) were found using the least-square minimization given by Sheppard, et al. [21]. The solid line of Fig. 5 is calculated from Eq. 2 using the least-square fitted Debye parameters.

The important electrical parameters which determine the interaction of electromagnetic radiation with human body, the attenuation coefficient and the emissivity, are shown in Figs. 6 and 7, respectively. Table 1 gives the Debye parameters for human skin in vivo from our measurement data (including 8 - 18 GHz data from Hey-Shipton, et al. [8]) and the Debye parameters for excised skin tissue from Cook [2]. In Figs. 6 and 7, the dashed line represents the calculated parameters from Debye dispersion for human skin in vivo while the solid line represents the calculated parameters from the Debye dispersion for excised skin tissue. Figure 8 shows the complex permittivity values calculated for human skin in vivo and excised skin tissue from their

Debye dispersions. The root-mean-square error (RMSE) for the experimental data of Fig. 5 has been found to be 0.62. The RMSE is defined by

$$RMSE = \left[\frac{\sum_{i=1}^{N} \left| \varepsilon^*_{ei} - \varepsilon^*_{di} \right|^2}{2N} \right]^{1/2} \qquad (3)$$

where ε^*_{ei} is the measured complex permittivity at ith frequency, ε^*_{di} is the calculated complex permittivity from single relaxation Debye dispersion at the ith frequency and N is the number of data points.

DISCUSSION AND CONCLUSIONS

From Fig. 8, it is observed that ε' and ε'' values for human skin in vivo are smaller than those obtained from excised human skin tissue. These differences in permittivity values result in significant changes in emissivity and attenuation coefficient at millimeter wavelengths. As shown in Fig. 6, the attenuation coefficients (α(dB/mm)) for human skin in vivo are lower than those obtained for the excised skin tissue. So, the skin depth (= 8.686 /α) for live skin is higher than that for the excised skin tissue. The emissivity (%) represents the percentage of power absorbed by the skin. From the results presented in Fig. 7, it is observed that the power coupling coefficient to the human body is somewhat higher than previously thought from Cook [2] and England [4] data for excised skin tissue.

In Table 1, the Debye parameters for excised skin tissue given by Cook [2] are compared with those obtained for human skin in vivo. It is seen that the dielectric behavior of human skin in vivo as well as

excised skin tissue is characterized by the relaxation of free water. Beside the expected differences between excised and live tissues regarding water, protein and salt content, it should be noted that the results for excised skin tissue are at 37°C while the live tissue results are at the ambient temperature (23°C). The difference in temperature could account for differences in relaxation time between live skin and excised skin tissue. The relaxation time of the water in live skin (9.205 ps) is somewhat higher than the relaxation time of pure water at 23°C (8.73 ps). This may be due to hydrogen bonding between water molecules and active groups of other types of molecules [2].

Schwan [7] has previously observed that the low frequency permittivity (ε_s) is determined by the total solid content of the tissue. Assuming dielectric decrement of 1 per gm solids in 100 c.c. [7], p which denotes percent solid content (gms/100 c.c.) is given by

$$\varepsilon_s = \varepsilon_{sw} - p \tag{4}$$

where ε_{sw} is the low frequency permittivity of water. From [22] ε_{sw} for water at 23°C is 78.56. So, the ε_{sw} of 25.44 for human skin <u>in vivo</u> corresponds to 53.12 percent solid content while the ε_s of 42 for excised skin tissue corresponds to 36.56 percent solid content. So, the reduction in ε_s for human skin <u>in vivo</u> as compared with excised skin tissue can be explained on the basis of solid content of the skin tissue.

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Table 1. Debye parameters for human skin *in vivo* and excised human skin tissue.

	ε_s	ε_∞	τ(ps)	σ(S/m)
Human skin *in vivo*	25.44 ± 1.2	1.5	9.205 ± 0.6	1.4
Excised human skin tissue from Cook [2]	42	4.0	6.9	1.4

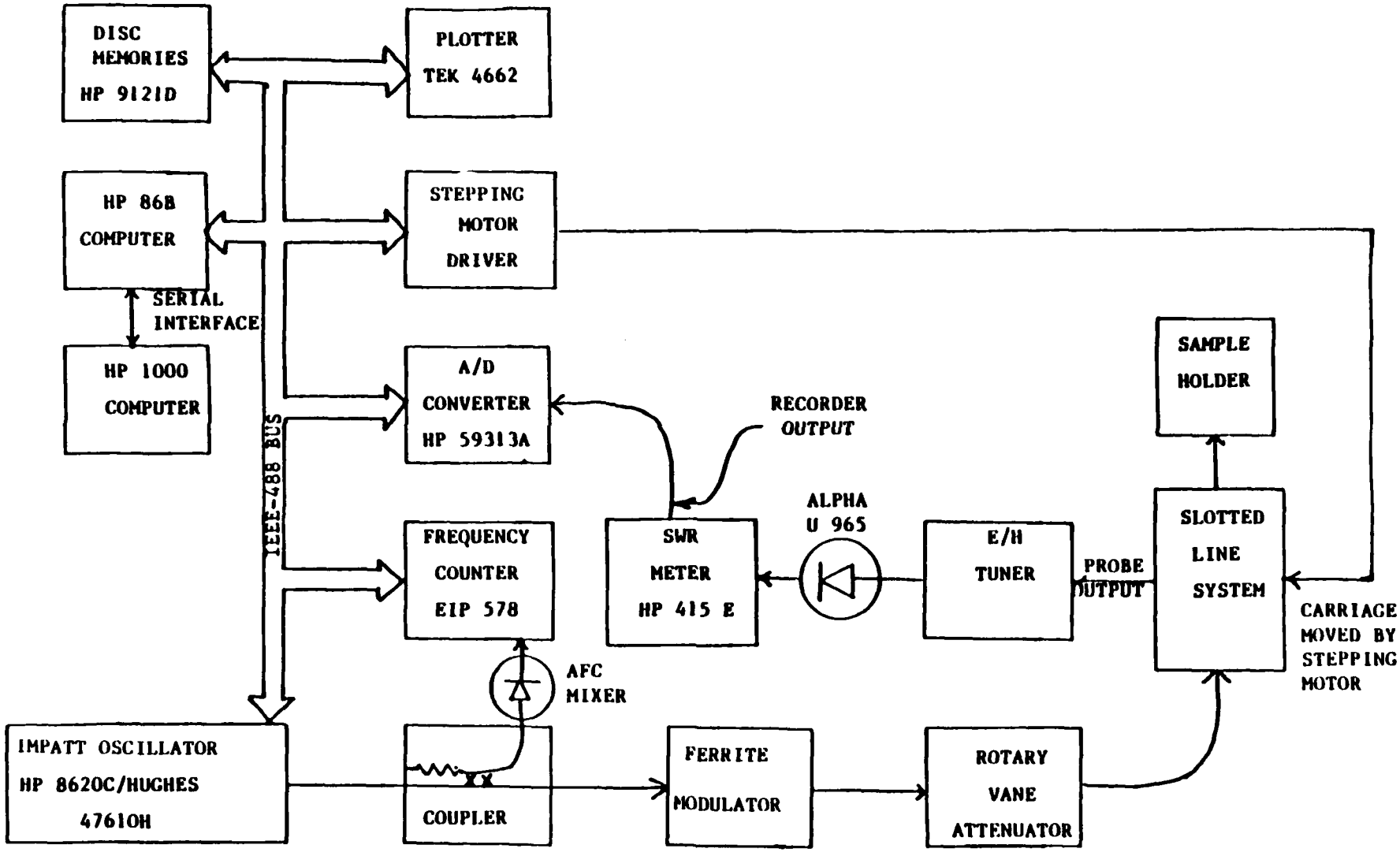


Fig. 1. Block diagram of automated measurement system for the frequency band 40 - 60 GHz. Similar measurement system has been developed for 26.5 - 40 GHz band.

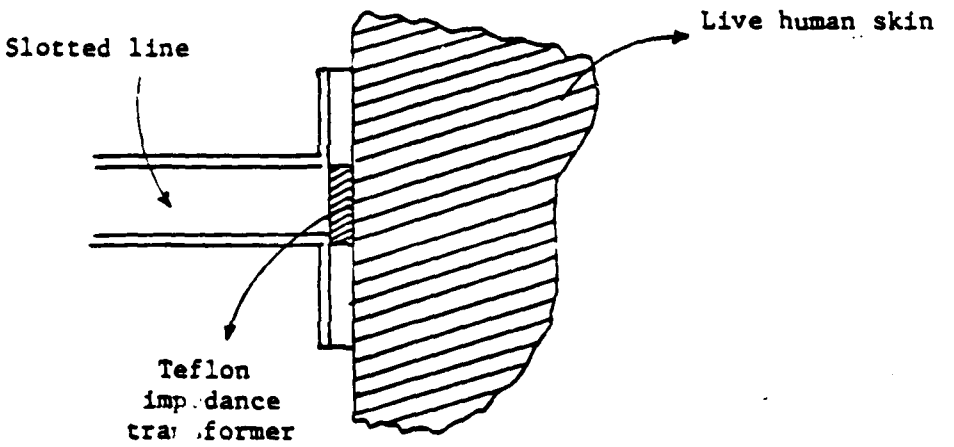


Fig. 2. Sample holder for in vivo measurements.

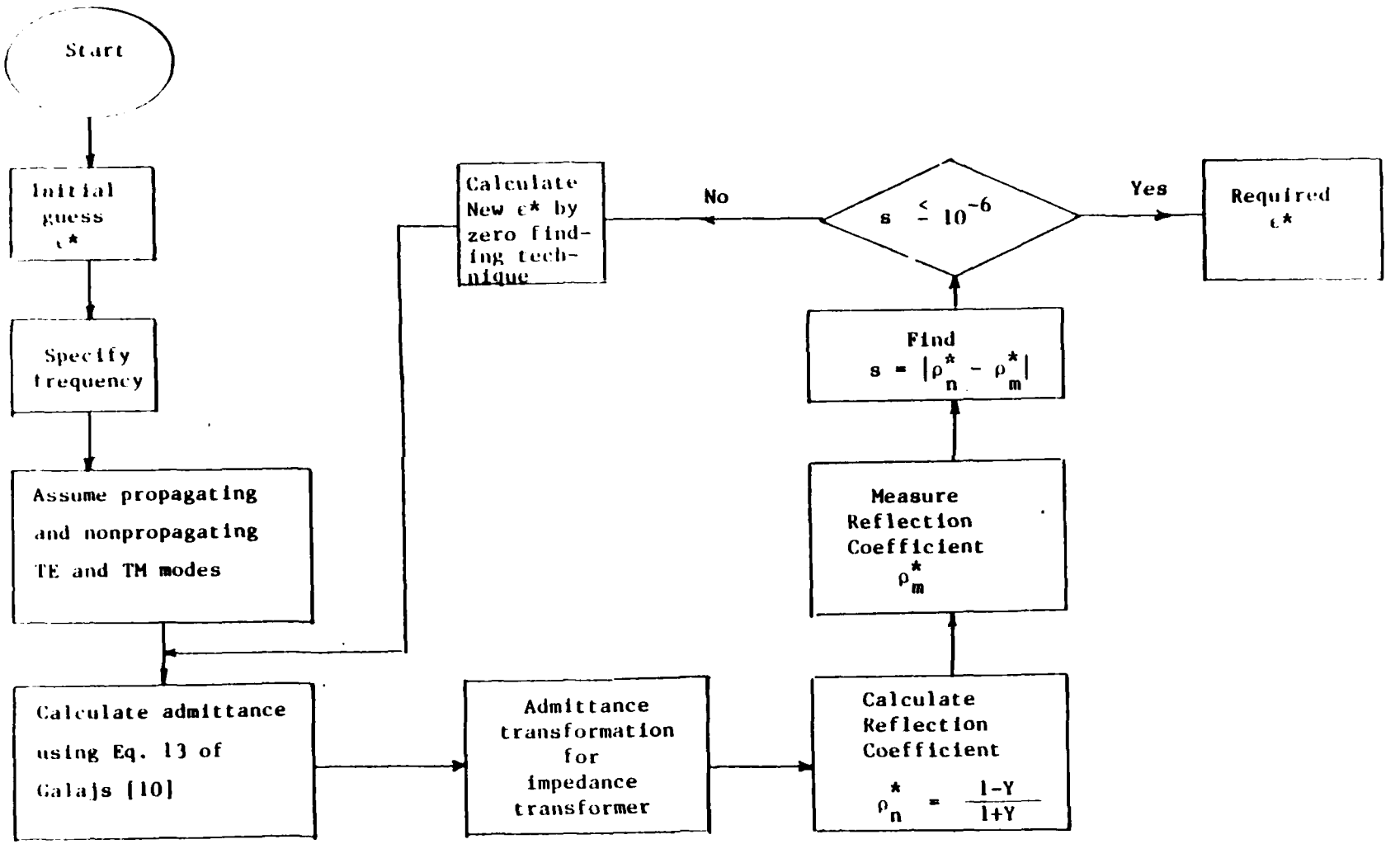
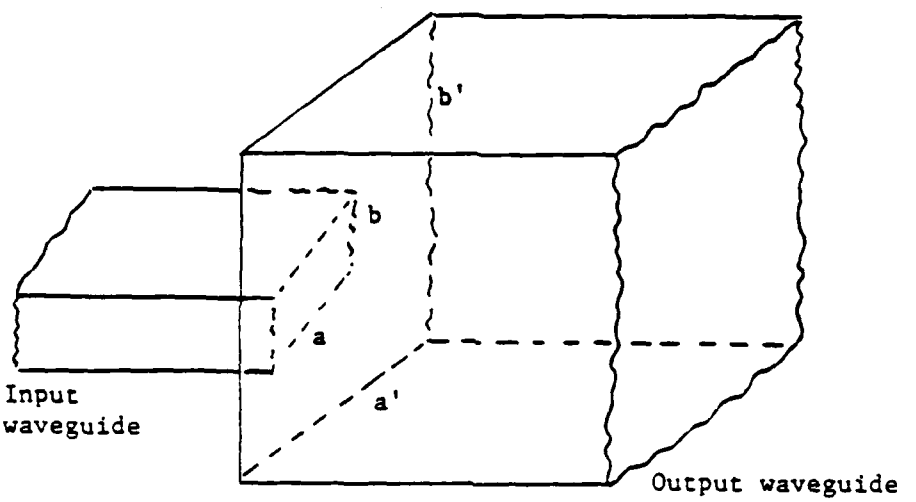


Fig. 3. Flow chart for in vivo dielectric measurements.



$a/b = 2.25, \qquad a'/a = 1, \qquad b'/b = 2, \qquad a = \lambda_o$

Fig. 4. Schematic diagram of input and output waveguides.

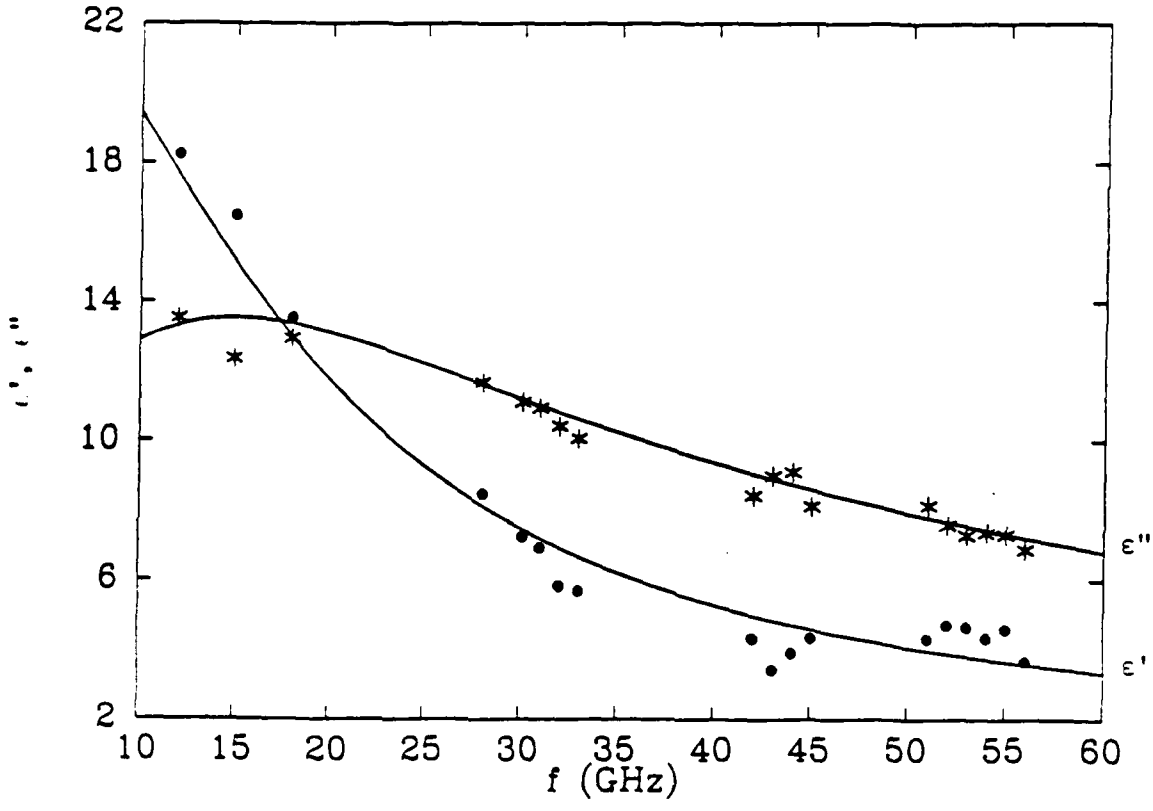


Fig. 5. Measured and calculated complex permittivities for human skin in vivo.

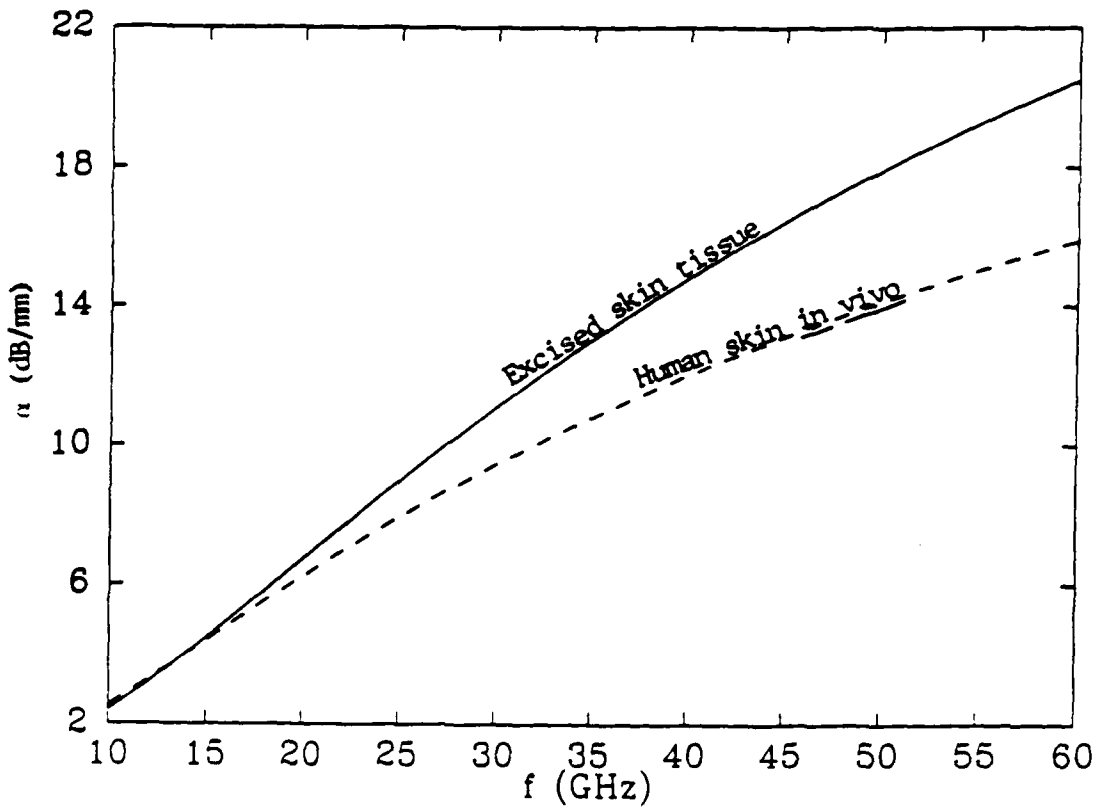


Fig. 6. Attenuation coefficient for the human skin in vivo (present results) and the excised human skin tissue (from Cook [2]).

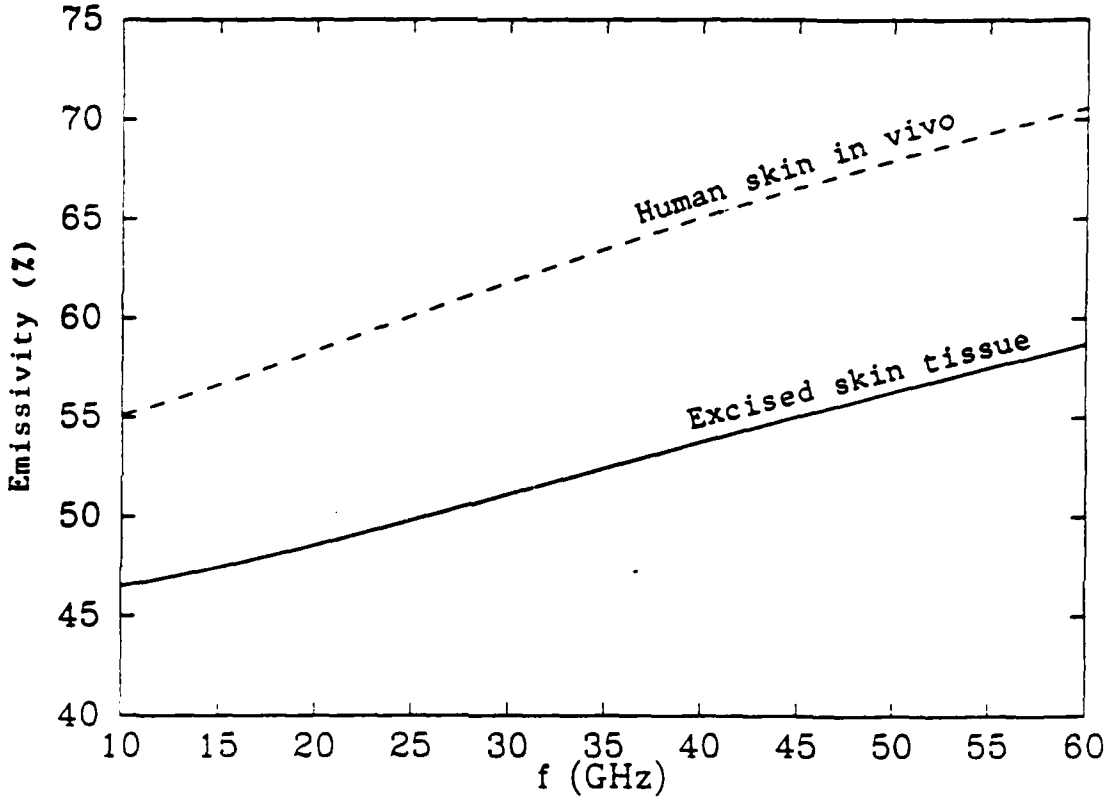


Fig. 7. Emissivity (percent) for the human skin in vivo (present results) and the excised skin tissue.

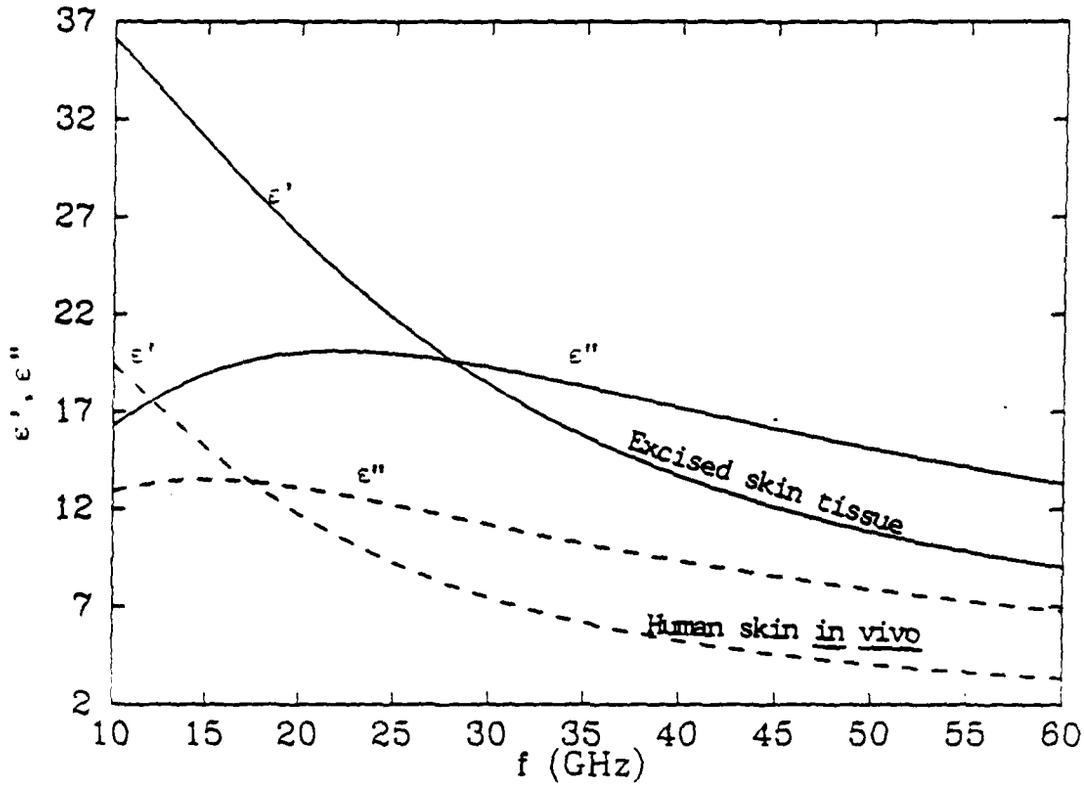


Fig. 8. Complex permittivity values for human skin in vivo (present results) and excised skin tissue.

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